

**FIELD OF THE INVENTION**

The present invention is related to nucleoside analogs for  
10 treating cancer, in particular dioxolane nucleoside  
analog.

**BACKGROUND OF THE INVENTION**

15 Neoplastic diseases, characterized by the proliferation of  
cells not subject to the normal control of cell growth,  
are a major cause of death in humans. In the United States  
only, a total of over about 1 million new cancer cases  
occurred for the year of 1995 (CA, Cancer J. Clin.,  
20 1995:45:8:30) cancer deaths in the United States for 1995  
was more than about 500,000.

The usefulness of known cytotoxic agents is compromised by  
dose limiting toxicities such as myelosuppression as well  
25 as the resistance of treated tumors. In view of the proven  
effectiveness of chemotherapy in the treatment of  
responsive tumors, efforts have been undertaken to develop  
novel compounds with either an improved therapeutic index  
or with reduced cross-resistance.

30 Antimetabolites, such as nucleoside analogs, have been  
used in anticancer treatment regimens. Some of the more  
commonly used analogs include gemcitabine (dFdC),

5 5-fluorouracil (5-FU), cytosine arabinoside (Ara-C,  
cytarabine), 6-thioguanine (TG) and 6-mercaptopurine (MP).  
This class of compounds is generally toxic to adult  
tissues that retain a high rate of cell proliferation:  
bone marrow, intestinal mucosa, hair follicles and gonads.

10

5-FU is used most commonly in breast and gastrointestinal  
cancer patients. Major side effects associated with 5-FU  
administration include bone marrow and mucous membrane  
toxicities; and minor side effects include skin rashes,  
15 conjunctivitis and ataxia. Ara-C, used in the treatment  
of acute myelocytic leukemia, may cause myelosuppression  
and gastrointestinal toxicity. TG and MP, used primarily  
in leukemia patients and rarely in solid tumors, are  
associated with toxicities similar to that of Ara-C.

20

$\beta$ -D-ddC has been investigated by Scanlon et al. in  
circumvention of human tumor drug resistance (WO  
91/07180). Human leukemia cells resistant to cisplatin  
have shown enhanced sensitivity to  $\beta$ -D-ddC. However,  
25  $\beta$ -D-ddC has been linked to the development of peripheral  
neuropathy (Yarchoan, et al, Lancet, i:76, 1988) and  
therefore exhibits in vivo toxicity.

More recently,  $\beta$ -L-Dioxolane cytidine (troxacitabine) was  
30 reported to demonstrate anticancer activity ( Grove et al.  
Cancer Research 55, 3008-3011, July 15 1995).

5

There is therefore a need for anticancer agents that are easy to synthesize and display an improved therapeutic index and efficacy against refractory tumors.

10 **SUMMARY OF THE INVENTION**

It is known that gemcitabine and cytarabine enter cancer cells by nucleoside or nucleobase transporter proteins.

Mackey et al., *supra*; White et al. (1987). *J. Clin.*

15 *Investig.* 79, 380-387; Wiley et al. (1982); *J. Clin.*

*Investig.* 69, 479-489; and Gati et al. (1997), *Blood* 90,

346-353. Further, it has been reported that troxacitabine also enters cancer cells by way of nucleoside or nucleobase transporter proteins (NTs). [Grove et al., *Cancer Research*

20 (56), p. 4187-91 (1996)] However, recent studies show

that troxacitabine actually enters cancer cells

predominately by the mechanism of passive diffusion,

rather than by nucleoside transporters. Cytarabine may

also enter cells by passive diffusion, but only during a

25 high-dose therapy regimen.

Also, resistance of cancer cells to treatment by

anticancer agents has been linked to a deficiency of

nucleoside or nucleobase transporter proteins in the cancer

30 cells. (Mackey et al. (1998), *supra*; Mackey et al.

(1998b). *Drug Resistance Updates* 1, 310-324; Ullman et

5 al. (1988), *J. Biol. Chem.* 263, 12391-12396; and  
references cited above.

Thus, in accordance with the invention, cancer treatments  
are provided in which the anticancer agents utilized enter  
10 cells by mechanisms other than through the use of  
nucleoside or nucleobase transporter proteins,  
particularly by passive diffusion. Transport through the  
cell membrane is facilitated by the presence of lipophilic  
structures. Thus, in accordance with the invention, entry  
15 of anticancer agents into cancer cells by passive  
diffusion is enhanced by providing the agents with  
lipophilic structures.

Further, in accordance with the invention, patients with  
20 cancers resistant to agents that are transported by  
nucleoside or nucleobase transporter proteins can be  
treated with anticancer agents that enter the cells  
predominately by passive diffusion.

25 Further, in accordance with the invention, patients with  
cancers resistant to agents that are transported by  
nucleoside or nucleobase transporter proteins can be  
treated with dosages of anticancer agents that increase  
the entry into the cells by passive diffusion.



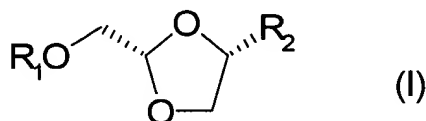
5 In accordance with one aspect of the invention, there is provided a method of treating a patient having a cancer which is resistant to gemcitabine, cytarabine, or both, by administering an anticancer agent that enters the cell predominately by a mechanism other than via nucleoside or  
10 nucleobase transporter proteins, particularly by passive diffusion. In the context of the invention, predominately means that the agent enters the cell by the specified mechanism to a greater degree than any one of the other individual transport mechanisms does.

15 In accordance with another aspect of the invention, there is provided a method of treating a patient having a cancer in which the cancer cells are deficient in nucleoside or nucleobase transporter proteins by administering an  
20 anticancer agent that enters the cell predominately by a mechanism other than via nucleoside or nucleobase transporter proteins, particularly that enter the cells predominately by passive diffusion.

25 In accordance with another aspect of the invention, there is provided a method of treating a patient having a cancer which is resistant to gemcitabine, cytarabine, and/or troxacitabine, by administering to the patient an anticancer agent, for example, a gemcitabine, cytarabine  
30 or troxacitabine derivative, that possesses a lipophilic structure to facilitate entry thereof into the cancer

5 cells, particularly by passive diffusion. In accordance  
 with another aspect of the invention, there is provided a  
 method of treating a patient having a cancer, which is  
 resistant to troxacitabine because of poor uptake, by  
 administering an anticancer agent, for example, a  
 10 troxacitabine derivative, which has a greater  
 lipophilicity than troxacitabine.

According to a further aspect of the invention, there is  
 provided a method for treating a patient having a cancer  
 15 that is resistant to gemcitabine and/or cytarabine  
 comprising administering to said patient a dioxolane  
 nucleoside compound of the following formula (I):



wherein:

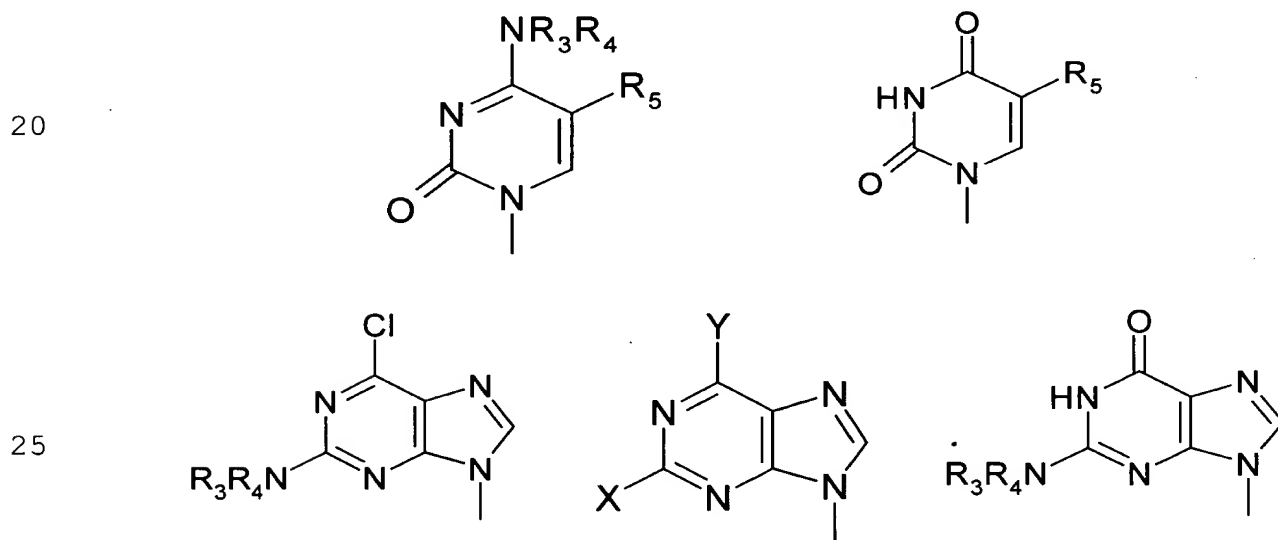
20  $R_1$  is  $H$ ;  $C_{1-24}$  alkyl;  $C_{2-24}$  alkenyl;  $C_{6-24}$  aryl;  
 trityl;  $C_{6-24}$ -aryl- $C_{1-24}$ -alkyl;  $C_{6-24}$ -aryl- $C_{2-24}$ -  
 alkenyl;  $C_{5-20}$  heteroaromatic ring;  
 25  $C_{3-20}$  non-aromatic ring optionally containing 1-3  
 heteroatoms selected from the group comprising  
 O, N, or S;  $-C(O)R_6$ ;  $-C(O)OR_6$ ;  $-C(O)NHR_6$ ; or an  
 amino acid radical or a dipeptide or tripeptide  
 chain or mimetic thereof, wherein the amino acid  
 30 radicals are selected from the group comprising  
 Glu, Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr,

5 Trp, Ser, Thr, Cys, Met, Asn and Gln (the amino acid chain preferably contains at least one amino acid other than Gly), and which in each case is optionally terminated by -R<sub>7</sub>;

10 R<sub>1</sub> can also be a P(O)(OR')<sub>2</sub> group wherein R' is in each case independently H, C<sub>1-24</sub> alkyl, C<sub>2-24</sub> alkenyl, C<sub>6-24</sub> aryl, C<sub>7-18</sub> arylmethyl, C<sub>2-18</sub> acyloxymethyl, C<sub>3-8</sub> alkoxy-carbonyloxymethyl, or C<sub>3-8</sub> S-acyl-2-thioethyl, saleginyl, t-butyl, phosphate or diphosphate;

15 R<sub>1</sub> can also be monophosphate, diphosphate, triphosphate or mimetics thereof;

R<sub>2</sub> is



30 R<sub>3</sub> and R<sub>4</sub> are in each case independently H; C<sub>1-24</sub> alkyl; C<sub>2-24</sub> alkenyl; C<sub>6-24</sub> aryl; C<sub>6-24</sub>-aryl-C<sub>1-24</sub>-alkyl; C<sub>6-24</sub>-aryl-C<sub>2-24</sub>-alkenyl; C<sub>5-18</sub> heteroaromatic ring;

5 C<sub>3-20</sub> non-aromatic ring optionally containing 1-3  
heteroatoms selected from the group comprising  
O, N, or S; -C(O)R<sub>6</sub>; -C(O)OR<sub>6</sub>; -C(O)NHR<sub>6</sub> or an  
amino acid radical or a dipeptide or tripeptide  
chain or mimetics thereof, wherein the amino  
10 acids radicals are selected from the group  
comprising Glu, Gly, Ala, Val, Leu, Ile, Pro,  
Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn and Gln  
(the amino acid chain preferably contains at  
least one amino acid other than Gly), and which  
15 in each case is optionally terminated by -R<sub>7</sub>;

R<sub>3</sub> and R<sub>4</sub> together can also be =CH-N(C<sub>1-4</sub>-alkyl)<sub>2</sub>;

R<sub>6</sub> is, in each case, H, C<sub>1-24</sub> alkyl, C<sub>2-24</sub> alkenyl,

C<sub>0-24</sub> alkyl-C<sub>6-24</sub> aryl, C<sub>6-24</sub>-aryl-C<sub>1-24</sub>-alkyl; C<sub>6-24</sub>-aryl-  
C<sub>2-24</sub>-alkenyl; C<sub>0-24</sub> alkyl-C<sub>5-20</sub> heteroaromatic ring, C<sub>3-20</sub>  
20 non-aromatic ring optionally containing 1-3 heteroatoms  
selected from the group comprising O, N or S;

R<sub>7</sub> is, in each case, C<sub>1-24</sub> alkyl, C<sub>2-24</sub> alkenyl, C<sub>6-24</sub>  
aryl, C<sub>6-24</sub>-aryl-C<sub>1-24</sub>-alkyl; C<sub>6-24</sub>-aryl-C<sub>2-24</sub>-alkenyl; C<sub>5-20</sub>  
heteroaromatic ring, C<sub>3-20</sub> non-aromatic ring optionally  
25 containing 1-3 heteroatoms selected from the group  
comprising O, N or S, -C(O)R<sub>6</sub> or -C(O)OR<sub>6</sub>; and

X and Y are each independently Br, Cl, I, F, OH, OR<sub>3</sub>  
or NR<sub>3</sub>R<sub>4</sub> and at least one of X and Y is NR<sub>3</sub>R<sub>4</sub>; or  
a pharmaceutically acceptable salt thereof.

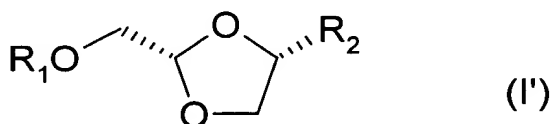
5 According to a further aspect of the invention, there is provided a method for treating a patient having a cancer that is resistant to gemcitabine, cytarabine and/or troxacitabine comprising administering to the patient a compound according to formula (I) wherein at least one of  
10  $R_1$ ,  $R_3$  and  $R_4$  is other than H, and if  $R_3$  and  $R_4$  are both H and  $R_1$  is  $-C(O)R_6$  or  $-C(O)OR_6$ , then  $R_6$  is other than H.

According to a further aspect of the invention, there is provided a method of treating a patient with cancer,  
15 wherein the cancer cells are deficient in one or more nucleoside or nucleobase transporter proteins, comprising administering to the patient a compound according to formula (I). According to a further aspect of the invention, there is provided a method for treating a patient with cancer, wherein the cancer cells are  
20 deficient in nucleoside or nucleobase transporter proteins, comprising administering to the patient a compound according to formula (I), wherein at least one of  $R_1$ ,  $R_3$  and  $R_4$  is other than H, and if  $R_3$  and  $R_4$  are both H and  $R_1$  is  $-C(O)R_6$  or  $-C(O)OR_6$ , then  $R_6$  is other than H.  
25

In accordance with another aspect of the invention, there is provided a method for treating a patient with cancer, comprising determining that a compound enters cancer cells  
30 predominately by passive diffusion, and administering the compound to the patient, wherein the compound is a

5 compound according to the formula (I). In accordance with another aspect of the invention, there is provided a method for treating a patient with cancer, comprising administering to the patient a compound which has been determined to enter cancer cells predominately by passive  
 10 diffusion, wherein the compound is in accordance with formula (I). In accordance with a further aspect of the invention, there is provided a method of treating a patient with cancer, comprising determining that a compound does not enter cancer cells predominately by  
 15 nucleoside or nucleobase transporter proteins, and administering the compound to the patient, wherein the compound is a compound according to the formula (I).

In accordance with an additional aspect of the invention  
 20 there are provided anticancer compounds having lipophilic structures, wherein the compounds are of the following formula (I'):



25

wherein:

$R_1$  is H;  $C_{1-24}$  alkyl;  $C_{2-24}$  alkenyl;  $C_{6-24}$  aryl;  
 trityl;  $C_{6-24}$ -aryl- $C_{1-24}$ -alkyl;  $C_{6-24}$ -aryl- $C_{2-24}$ -  
 30 alkenyl;  $C_{5-20}$  heteroaromatic ring;  
 $C_{3-20}$  non-aromatic ring optionally containing 1-3

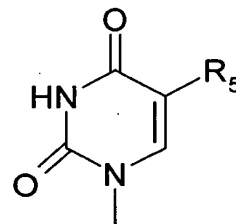
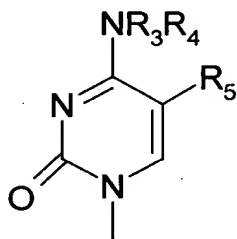
5 heteroatoms selected from the group comprising  
 O, N, or S;  $-C(O)R_6$ ;  $-C(O)OR_6$ ;  $-C(O)NHR_6$ ; or an  
 amino acid radical or a dipeptide or tripeptide  
 chain or mimetic thereof, wherein the amino acid  
 radicals are selected from the group comprising  
 10 Glu, Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr,  
 Trp, Ser, Thr, Cys, Met, Asn and Gln (the amino  
 acid chain preferably contains at least one  
 amino acid other than Gly), and which in each  
 case is optionally terminated by  $-R_7$ ;

15  $R_1$  can also be a  $P(O)(OR')_2$  group wherein  $R'$  is in  
 each case independently H,  $C_{1-24}$  alkyl,  $C_{2-24}$   
 alkenyl,  $C_{6-24}$  aryl,  $C_{7-18}$  arylmethyl,  $C_{2-18}$   
 acyloxymethyl,  $C_{3-8}$  alkoxy carbonyloxymethyl, or  
 $C_{3-8}$  S-acyl-2-thioethyl, saleginyl, t-butyl,  
 20 phosphate or diphosphate;

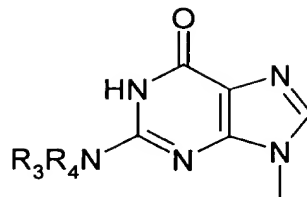
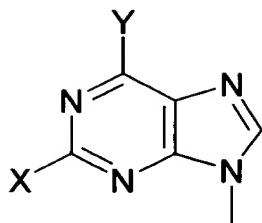
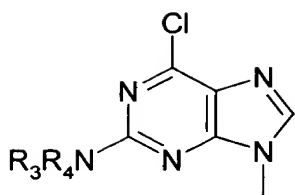
$R_1$  can also be monophosphate, diphosphate,  
 triphosphate or mimetics thereof;

$R_2$  is

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$R_3$  and  $R_4$  are in each case independently H;  $C_{1-24}$  alkyl;  $C_{2-24}$  alkenyl;  $C_{6-24}$  aryl;  $C_{6-24}$ -aryl- $C_{1-24}$ -alkyl;  $C_{6-24}$ -aryl- $C_{2-24}$ -alkenyl;  $C_{5-18}$  heteroaromatic ring;  $C_{3-20}$  non-aromatic ring optionally containing 1-3 heteroatoms selected from the group comprising O, N, or S;  $-C(O)R_6$ ;  $-C(O)OR_6$ ;  $-C(O)NHR_6$  or an amino acid radical or a dipeptide or tripeptide chain or mimetics thereof, wherein the amino acids radicals are selected from the group comprising Glu, Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn and Gln (the amino acid chain preferably contains at least one amino acid other than Gly), and which in each case is optionally terminated by  $-R_7$ ;

25

$R_3$  and  $R_4$  together can also be  $=CH-N(C_{1-4}\text{-alkyl})_2$ ;

30

$R_6$  is, in each case, H,  $C_{1-24}$  alkyl,  $C_{2-24}$  alkenyl,  $C_{0-24}$  alkyl- $C_{6-24}$  aryl,  $C_{6-24}$ -aryl- $C_{1-24}$ -alkyl;  $C_{6-24}$ -aryl- $C_{2-24}$ -alkenyl;  $C_{0-24}$  alkyl- $C_{5-20}$  heteroaromatic ring,  $C_{3-20}$  non-aromatic ring optionally containing 1-3 heteroatoms selected from the group comprising O, N or S;



5         $R_7$  is, in each case,  $C_{1-24}$  alkyl,  $C_{2-24}$  alkenyl,  $C_{6-24}$  aryl,  $C_{6-24}$ -aryl- $C_{1-24}$ -alkyl;  $C_{6-24}$ -aryl- $C_{2-24}$ -alkenyl;  $C_{5-20}$  heteroaromatic ring,  $C_{3-20}$  non-aromatic ring optionally containing 1-3 heteroatoms selected from the group comprising  
 10        O, N or S,  $-C(O)R_6$  or  $-C(O)OR_6$ ; and  
       X and Y are each independently Br, Cl, I, F, OH,  $OR_3$  or  $NR_3R_4$  and at least one of X and Y is  $NR_3R_4$ ; or a pharmaceutically acceptable salt thereof.  
       X and Y are each independently Br, Cl, I, F, OH,  $OR_3$   
 15        or  $NR_3R_4$  and at least one of X and Y is  $NR_3R_4$ ; or a pharmaceutically acceptable salt thereof;  
       with the proviso that at least one of  $R_1$ ,  $R_3$  and  $R_4$  is  
        $C_{7-24}$  alkyl;  
 20         $C_{7-24}$  alkenyl;  
        $C_{6-24}$  aryl;  
        $C_{5-20}$  heteroaromatic ring;  
        $C_{4-20}$  non-aromatic ring optionally containing 1-3 heteroatoms selected from the group comprising O, N, or S;  
 25         $-C(O)R_6$  in which  $R_6$  is ,  $C_{7-24}$  alkyl,  $C_{7-24}$  alkenyl,  $C_{0-24}$  alkyl- $C_{6-24}$  aryl,  $C_{6-24}$ -aryl- $C_{1-24}$ -alkyl;  $C_{6-24}$ -aryl- $C_{2-24}$ -alkenyl;  $C_{0-24}$  alkyl- $C_{5-20}$  heteroaromatic ring,  $C_{3-20}$  non-aromatic ring optionally containing 1-3 heteroatoms selected from the group comprising O, N or S ;  
 30         $-C(O)OR_6$  in which  $R_6$  is  $C_{7-24}$  alkyl,  $C_{7-24}$  alkenyl,  $C_{0-24}$  alkyl- $C_{6-24}$  aryl,  $C_{6-24}$ -aryl- $C_{1-24}$ -alkyl;  $C_{6-24}$ -aryl- $C_{2-24}$ -

5 alkenyl; C<sub>0-24</sub> alkyl-C<sub>5-20</sub> heteroaromatic ring, C<sub>3-20</sub>  
non-aromatic ring optionally containing 1-3 heteroatoms  
selected from the group comprising O, N or S; or

a dipeptide or tripeptide or mimetic thereof  
where the amino acid radicals are selected from the group  
10 comprising Glu, Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr,  
Trp, Ser, Thr, Cys, Met, Asn and Gln (and the amino acid  
chain preferably contains at least one amino acid other  
than Gly), and which is optionally terminated by -R<sub>7</sub>.

15 In an embodiment of the present invention, the R<sub>6</sub> group is  
connected to the rest of the molecule at a tertiary or  
quaternary carbon. A tertiary carbon is defined as a  
carbon atom which has only one hydrogen atom directly  
attached to it. A quaternary carbon is defined as a carbon  
20 atom with no hydrogen atoms attached to it.

In an alternate embodiment of the present invention, the R<sub>6</sub>  
group is selected as to provide steric hindrance in the  
vicinity of the carbonyl group.

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Upon further study of the specification and claims,  
further aspects and advantages of the invention will  
become apparent to those skilled in the art.

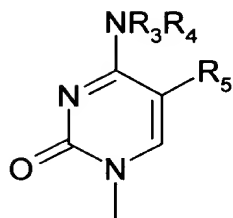
30 As mentioned above, recent studies have shown that  
troxacitabine, a L-nucleoside analog, enters cancer cells

5 predominately by passive diffusion, rather than by  
nucleoside or nucleobase transporter proteins. While this  
invention is not intended to be limited by any theoretical  
explanation, it is believed that this property of  
troxacitabine is at least in part attributed to the  
10 dioxolane structure. Further, due to its L-configuration,  
troxacitabine is a poor substrate for deoxycytidine  
deaminase. (Grove et al. (1995), *Cancer Res.* 55, 3008-  
3011) Formula (I) encompasses compounds which are  
nucleoside analogs having a dioxolane structure and which  
15 exhibit the L-configuration. In addition, formula (I)  
encompasses compounds which exhibit a lipophilic  
structure. In the case of compounds encompassed by  
formula (I), the lipophilic structures are provided  
through modification of the hydroxymethyl structure of the  
20 dioxolane sugar moiety and/or modification of amino groups  
of the base moiety.

In the compounds of formula (I), preferably at least one  
of  $R^1$ ,  $R^3$  and  $R^4$  provides a lipophilic structure. Thus,  
25 preferably at least one of  $R^1$ ,  $R^3$  and  $R^4$  is other than H  
and, if  $R^3$  and  $R^4$  are each H and  $R^1$  is  $C(O)R^6$ ,  $C(O)OR^6$  or  
 $C(O)NHR^6$  then  $R^6$  is other than H.

$R^2$  is preferably a cytosine base structure, as in the case  
30 of troxacitabine. In particular,  $R^2$  is preferably

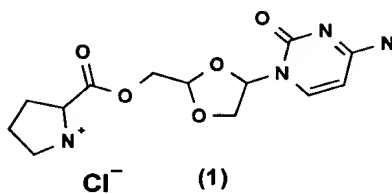
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15 The following are examples of compounds in accordance with the invention:

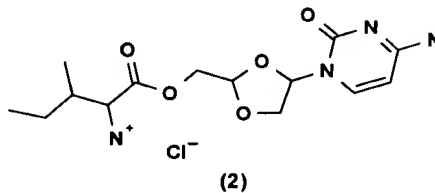
COMPOUND #1



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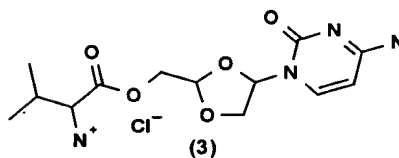
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COMPOUND #2



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COMPOUND #3

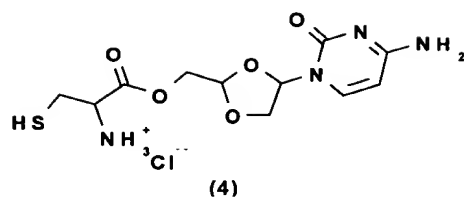


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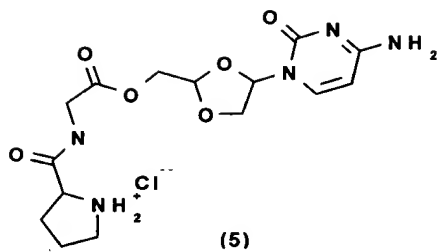
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COMPOUND #4

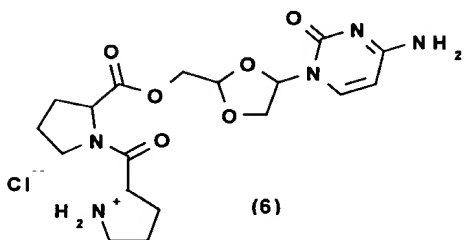
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10 COMPOUND #5

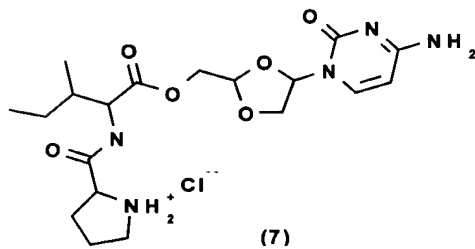


COMPOUND #6



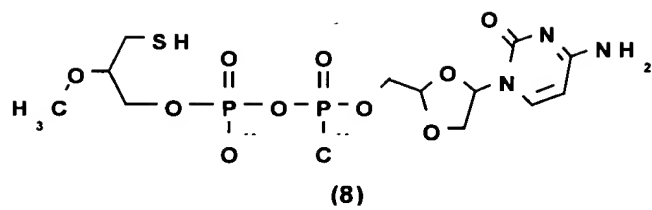
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COMPOUND #7



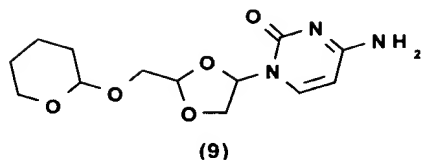
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## 5 COMPOUND #8



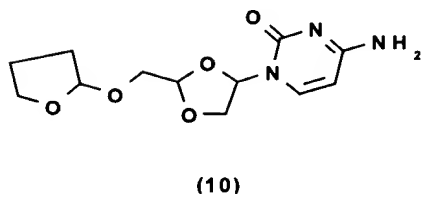
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## COMPOUND #9



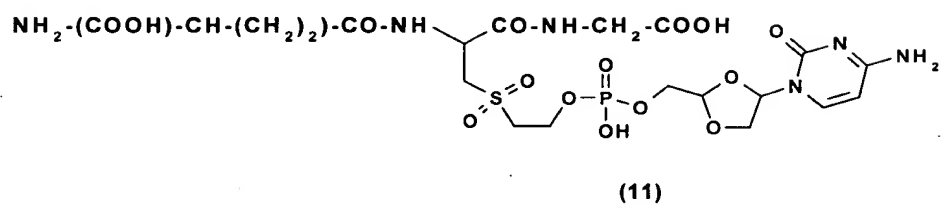
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## COMPOUND #10

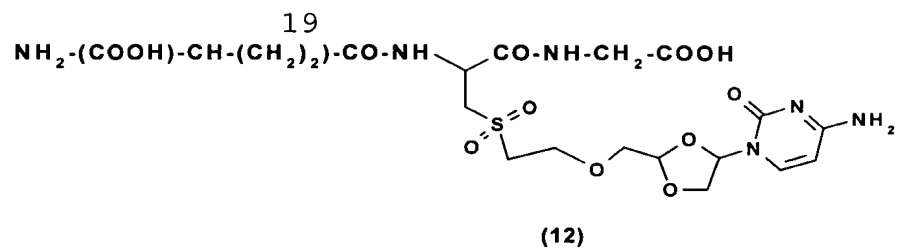


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## COMPOUND #11

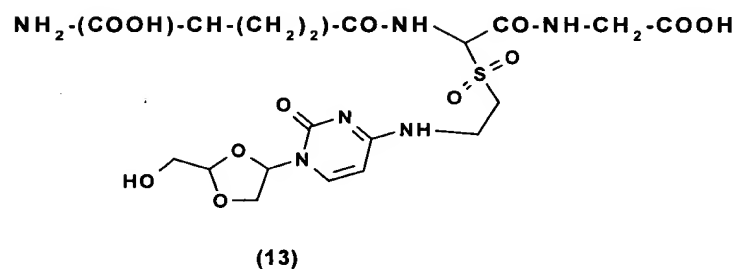


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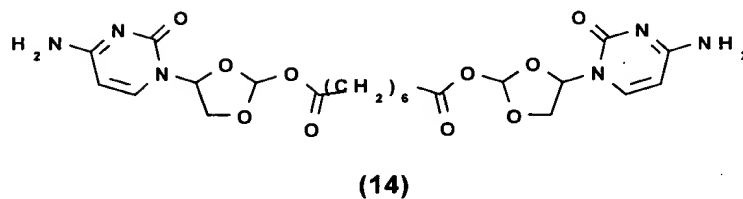
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COMPOUND #13



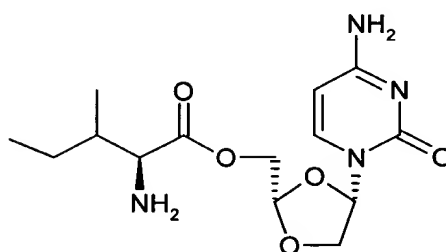
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COMPOUND #14



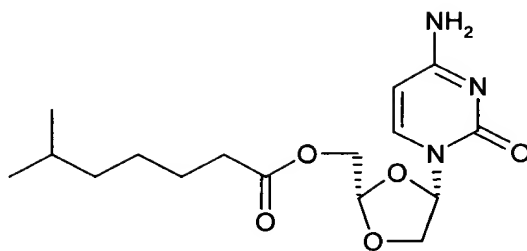
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COMPOUND #15



COMPOUND #16

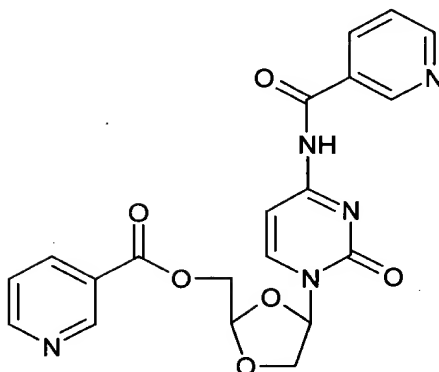
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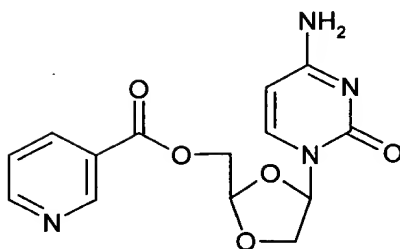
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COMPOUND #17



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COMPOUND #18

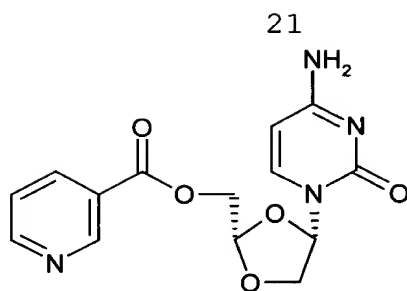


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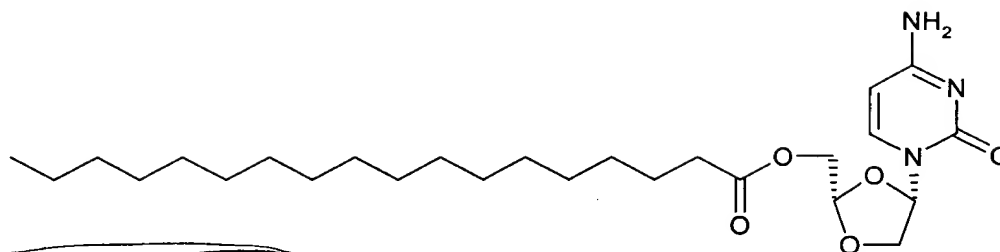
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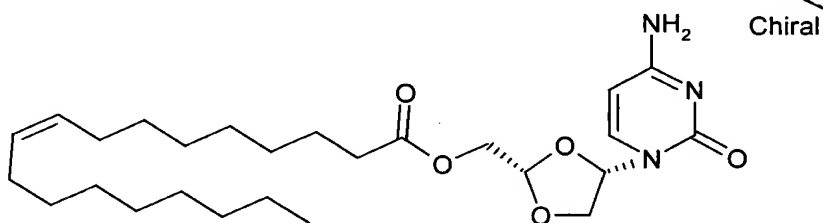
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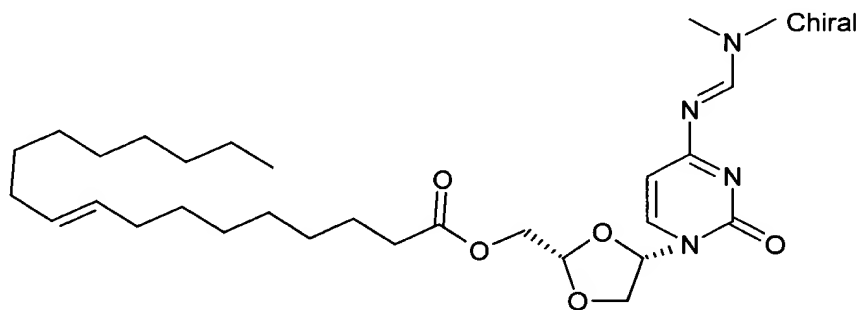


COMPOUND #21



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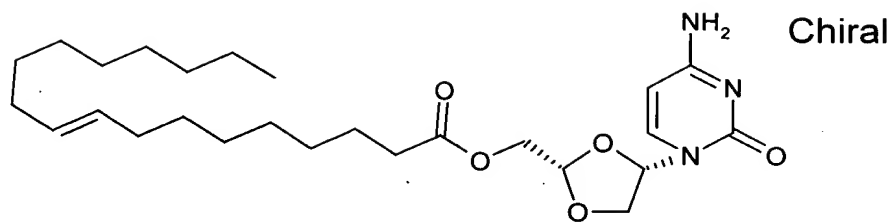
COMPOUND #22



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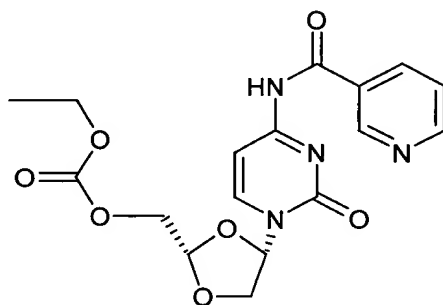
COMPOUND #23

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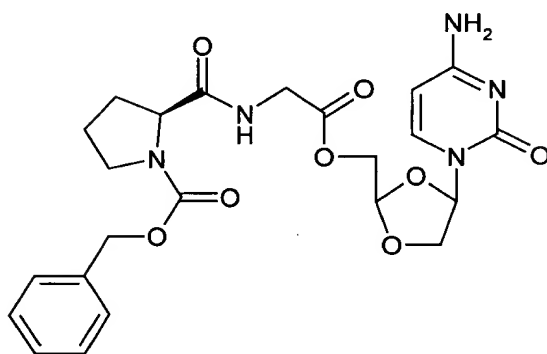
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COMPOUND #24

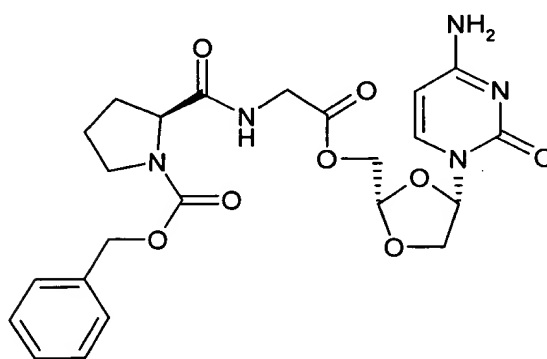


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COMPOUND #25

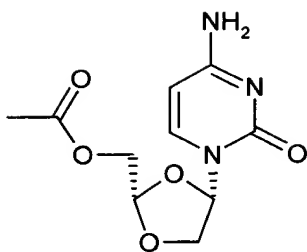


COMPOUND #26



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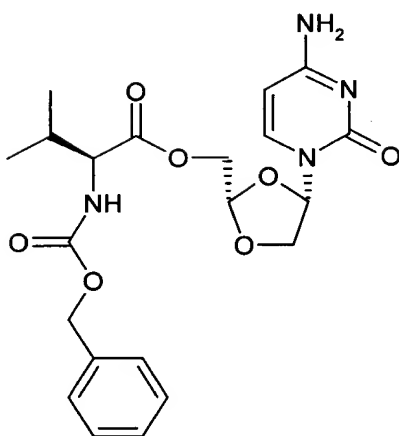
## 5 COMPOUND #27



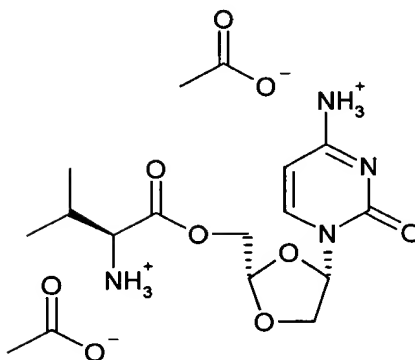
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## 15 COMPOUND #28

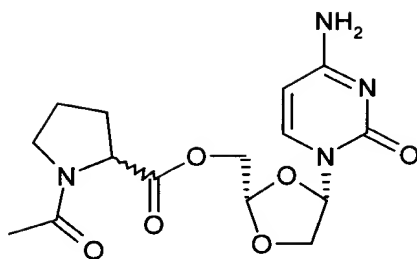


## COMPOUND #29



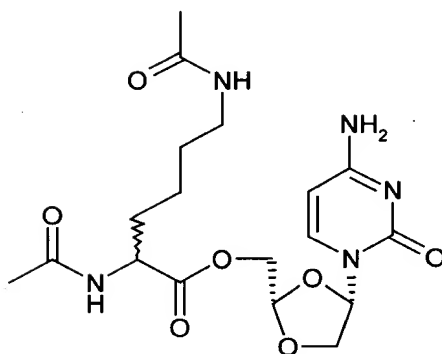
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COMPOUND #30



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15 COMPOUND #31



COMPOUND #32

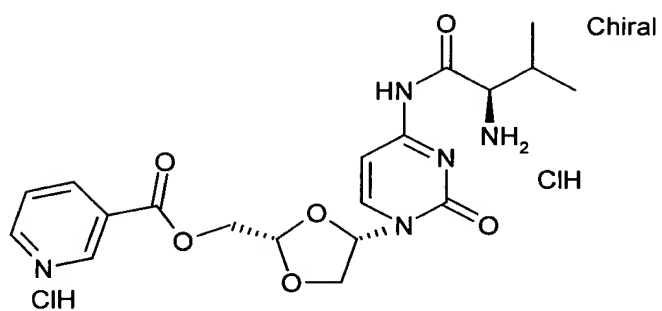
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Chiral

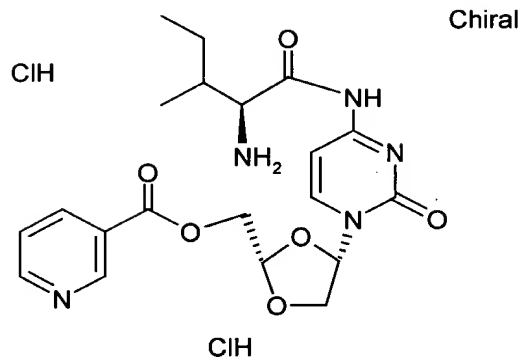


## 5 COMPOUND #35



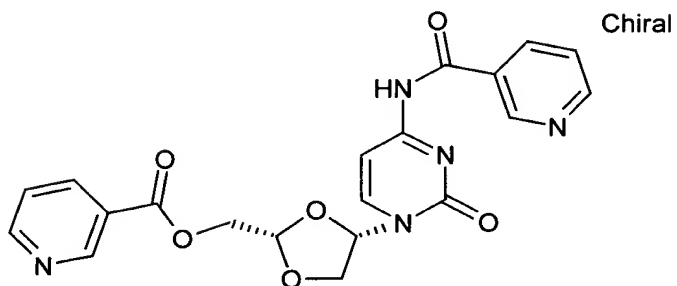
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## COMPOUND #36



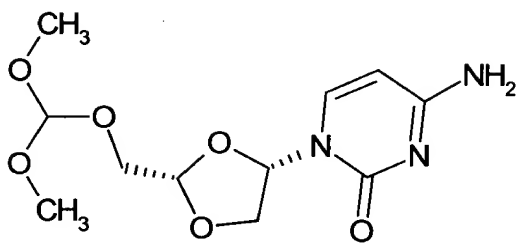
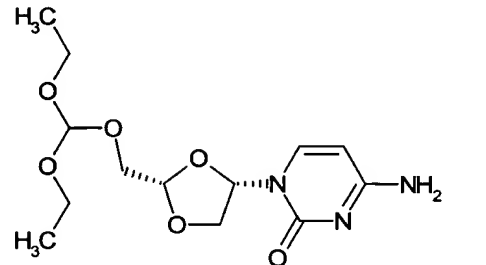
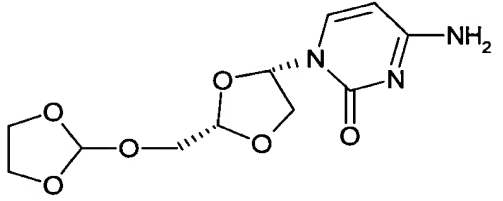
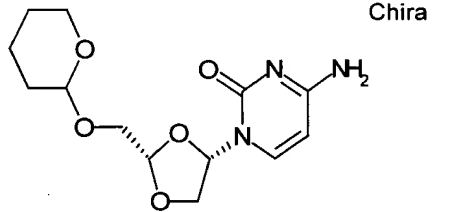
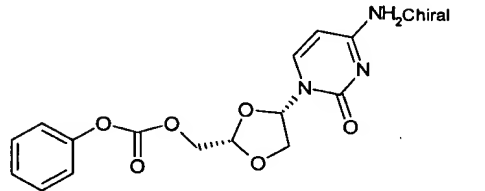
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## COMPOUND #37



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- 5 The following compounds 38 to 281 are also compounds in accordance with the invention:

No.	Name	Structure
38	4-AMINO-1-(2-DIMETHOXYMETHOXYMETHYL-[1,3]DIOXOLAN-4-YL)-1H-PYRIMIDIN-2-ONE	
39	4-AMINO-1-(2-DIETHOXYMETHOXYMETHYL-[1,3]DIOXOLAN-4-YL)-1H-PYRIMIDIN-2-ONE	
40	4-AMINO-1-[2-([1,3]DIOXOLAN-2-YLOXYMETHYL)-[1,3]DIOXOLAN-4-YL]-1H-PYRIMIDIN-2-ONE	
41	4-AMINO-1-[2-(TETRAHYDRO-PYRAN-2-YLOXYMETHYL)-[1,3]DIOXOLAN-4-YL]-1H-PYRIMIDIN-2-ONE	
42	CARBONIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER PHENYL ESTER	

No.	Name	Structure	
43	CARBONIC ACID 4-(2-OXO-4-PHENOXYCARBONYLAMINO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER PHENYL ESTER		Chiral
44	[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-CARBAMIC ACID PHENYL ESTER		Chiral
45	[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-CARBAMIC ACID ETHYL ESTER		Chiral
46	CARBONIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER ETHYL ESTER		Chiral
47	CARBONIC ACID 4-(4-ETHOXYCARBONYLAMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER ETHYL ESTER		Chiral

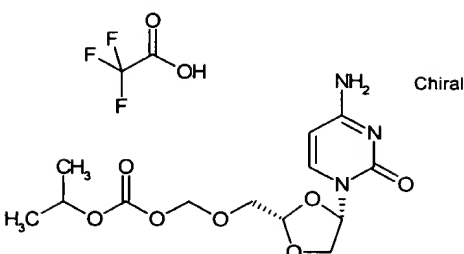
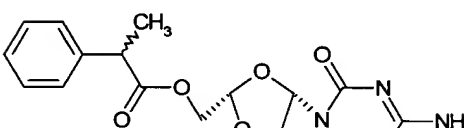
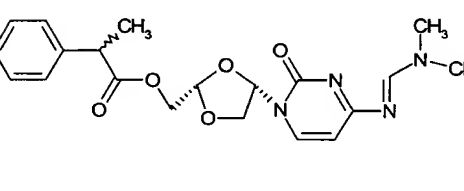
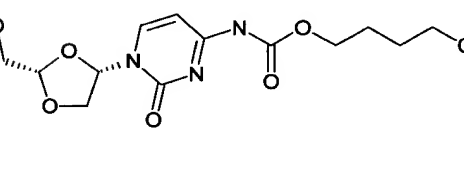
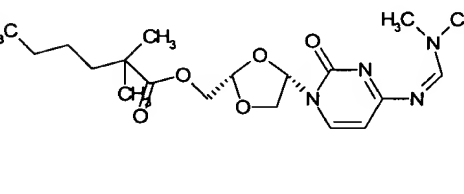


No.	Name	Structure	Chiral
48	BUTYL-CARBAMIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER		Chiral
49	N-[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-CYTOSYL]-2,2-DIMETHYL-PROPIONAMIDE		
50	[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-CYTOSYL]-CARBAMIC ACID BENZYL ESTER		
51	4-(4-BENZYLOXYCARBONYLAMINOCYTOSYL)-[1,3]DIOXOLAN-2-YLMETHYL BENZYL CARBONATE		
52	(2S,4S)-2-PHENYLACETOXYMETHYL-4-CYTOSIN-1'-YL-1,3-DIOXOLANE		

No.	Name	Structure
53	4-AMINO-1-(2-TRITYLOXYMETHYL-[1,3]DIOXOLAN-4-YL)-1H-PYRIMIDIN-2-ONE	
54	4-AMINO-1-[2-(1-METHOXY-1-METHYLETHOXYMETHYL)-[1,3]DIOXOLAN-4-YL]-1H-PYRIMIDIN-2-ONE	
55	OCTANOIC ACID [1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-AMIDE	
56	4-AMINO-1-(2-BENZYLOXYMETHOXYMETHYL-[1,3]DIOXOLAN-4-YL)-1H-PYRIMIDIN-2-ONE	
57	CARBONIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER BENZYL ESTER	

No.	Name	Structure
58	2,2-DIMETHYL-PROPIONIC ACID 4-(4-AMINO-2-OXO- 2H-PYRIMIDIN-1-YL)- [1,3]DIOXOLAN-2- YLMETHOXYMETHYL ESTER	
59	[1-(2-HYDROXYMETHYL- [1,3]DIOXOLAN-4-YL)-2- OXO-1,2-DIHYDRO- PYRIMIDIN-4-YL]- CARBAMIC ACID BUTYL ESTER	
60	(2S,4S)--2- HYDROXYMETHYL-4-N-[2''- (2''-NITROPHENYL)-2''- METHYLPROPIONYL]- CYTOSINE-1'-YL-1,3- DIOXOLANE	
61	[1-(2-HYDROXYMETHYL- [1,3]DIOXOLAN-4-YL)-2- OXO-1,2-DIHYDRO- PYRIMIDIN-4-YL]- CARBAMIC ACID HEXYL ESTER	
62	4-AMINO-1-[2-(2- METHOXY- ETHOXYMETHOXYMETHYL)- [1,3]DIOXOLAN-4-YL]-1H- PYRIMIDIN-2-ONE	

No.	Name	Structure
63	CARBONIC ACID 4-[4-(4-METHOXY-PHENOXYCARBONYLAMINO)-2-OXO-2H-PYRIMIDIN-1-YL]-[1,3]DIOXOLAN-2-YLMETHYL ESTER 4-METHOXY-PHENYL ESTER	
64	(2S,4S)-2-(2''-METHYL-HEXANOICOXYMETHYL)-4-(4'-NN-DIMETHYLAMINOMETHYLENE-CYTOSIN-1'-YL)-1,3-DIOXOLANE	
65	(2S,4S)-2-(2''-ETHYL-HEXANOICOXYMETHYL)-4-(4'-N,N-DIMETHYLAMINOMETHYLENE-CYTOSIN-1'-YL)-1,3-DIOXOLANE	
66	6-(Benzyl-tert-butoxycarbonyl-amino)-hexanoic acid 4-(4-amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethyl ester	
67	CARBONIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER ISOPROPYL ESTER TRIFLUOROACETATE SALT	

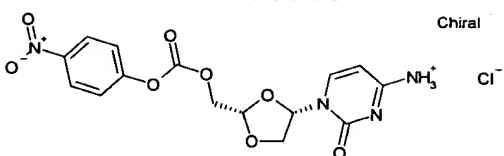
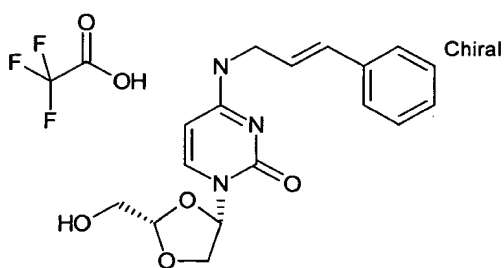
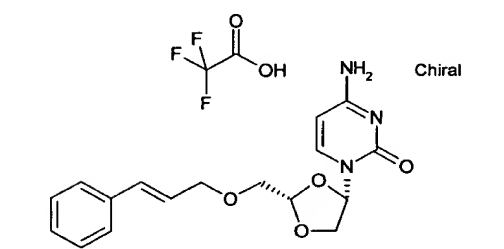
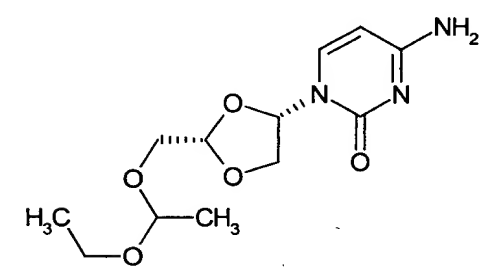
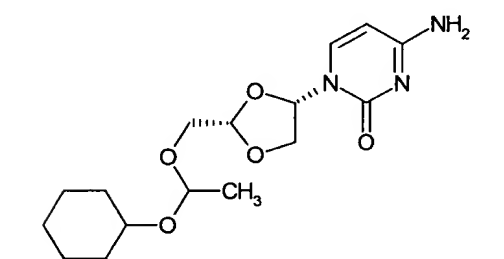
No.	Name	Structure
68	CARBONIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHOXYMETHYL ESTER ISOPROPYL ESTER TRIFLUOROACETIC ACID SALT	
69	(2S,4S)-2-(2''-METHYLPHENYLACETOXY)METHYL-4-CYTOSIN-1'-YL-1,3-DIOXOLANE	
70	(2S,4S)-2-(2''-METHYLPHENYLACETOXY)METHYL-4-(4'-N,N-DIMETHYLAMINOMETHYLENE-CYTOSIN-1'-YL)-1,3-DIOXOLANE	
71	[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-CARBAMIC ACID PENTYL ESTER	
72	(2S,4S)-2-(2''-DIMETHYLHEXANOICOXYMETHYL)-4-(4'-N,N-DIMETHYLAMINOMETHYLENE-CYTOSIN-1'-YL)-1,3-DIOXOLANE	

No.	Name	Structure
73	[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-CARBAMIC ACID 4-METHOXY-PHENYL ESTER	
74	1-(2-ALLYLOXYMETHYL-[1,3]DIOXOLAN-4-YL)-4-AMINO-1H-PYRIMIDIN-2-ONE	
75	4-AMINO-1-(2(S)-ETHOXYMETHYL-[1,3]DIOXOLAN-4(S)-YL)-1H-PYRIMIDIN-2-ONE	
76	N-[1-(2(S)-D-RIBOSYLOXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-ACETAMIDE	
77	Benzyl-{5-[1-(2-hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-ylcarbamoyl]-pentyl}-carbamic acid tert-butyl ester	

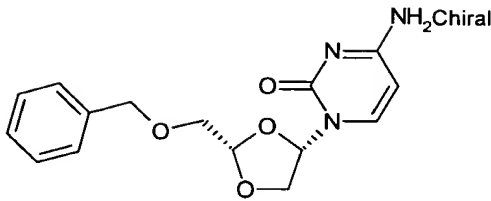
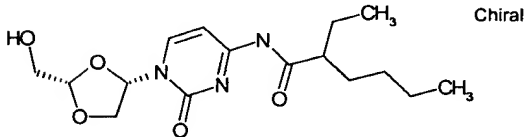
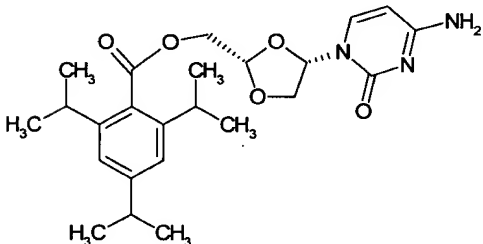
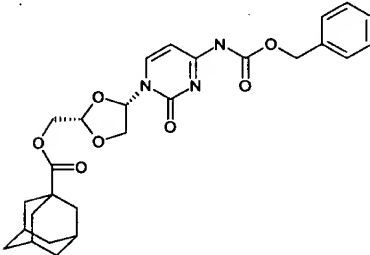
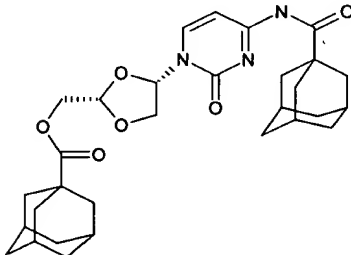
No.	Name	Structure
78	6-(Benzyl-tert-butoxycarbonyl-amino)-hexanoic acid 4-{4-[6-(benzyl-tert-butoxycarbonyl-amino)-hexanoylamino]-2-oxo-2H-pyrimidin-1-yl}-[1,3]dioxolan-2-ylmethyl ester	
79	2,2,2-TRICHLORO-ACETIMIDIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER	
80	PENTANEDIOIC ACID 4-[4-(4-METHOXYCARBONYLBUTYRYLAMINO)-2-OXO-2H-PYRIMIDIN-1-YL]-[1,3]DIOXOLAN-2-YLMETHYL ESTER METHYL ESTER	
81	4-[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YLCARBAMOYL]-BUTYRIC ACID METHYL ESTER	
82	PENTANEDIOIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER METHYL ESTER	

No.	Name	Structure
83	6-Benzylamino-hexanoic acid 4-(4-amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethyl ester bis trifluoroacetate salt	
84	6-Benzylamino-hexanoic acid 4-(4-amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethyl ester	
85	4-AMINO-1-[2-(3,4-DIHYDROXY-5-HYDROXYMETHYL-TETRAHYDROFURAN-2-YLOXYMETHYL)-[1,3]DIOXOLAN-4-YL]-1HPYIMIDIN-2-ONE, TRIFLUOROACETIC ACID SALT	<div data-bbox="1305 785 1354 814" style="position: absolute; left: 80.4%; top: 37.4%;">Chiral</div>
86	(2S,4S)-2-(2"-METHYL-HEXANOICOXYMETHYL)-4-CYTOSIN-1'-YL-1,3-DIOXOLANE HYDROCHLORIDE	<div data-bbox="1370 1108 1403 1138" style="position: absolute; left: 84.4%; top: 52.8%;">Cl<sup>-</sup></div>
87	(2S,4S)-2-(2",6"-DIMETHYLBENZOYLOXYMETHYL)-4-(4'-N,N-DIMETHYLAMINOMETHYLENE-CYTOSIN-1'-YL)-1,3-DIOXOLANE	

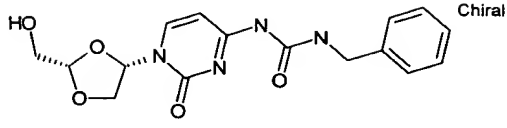
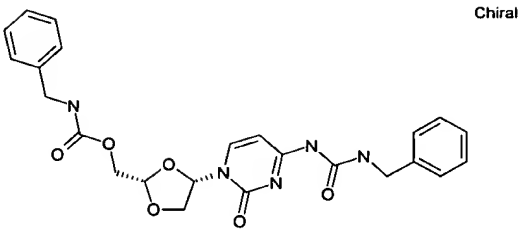
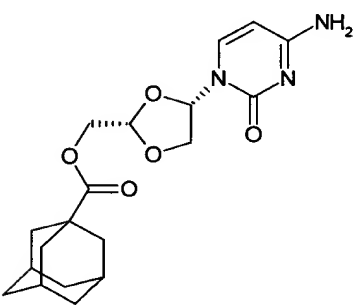
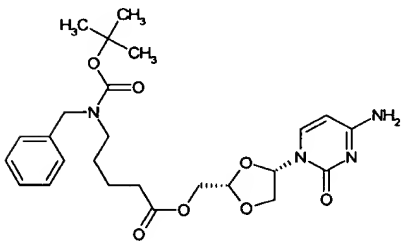
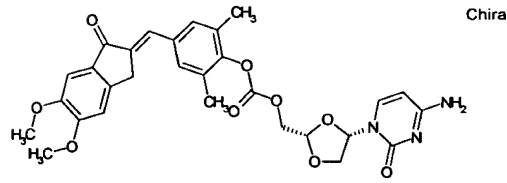


No.	Name	Structure
88	1-[2-(4-NITRO-PHENOXYCARBOXYLOXYMETHYL)-[1,3]DIOXOLAN-4-YL]-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL-AMMONIUM; CHLORIDE	
89	1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-4-(3-CINNAMYL)-1H-PYRIMIDIN-2-ONE TRIFLUORO-ACETATE SALT	
90	4-AMINO-1-[2-(3-CINNAMYLOXYMETHYL)-[1,3]DIOXOLAN-4-YL]-1H-PYRIMIDIN-2-ONE TRIFLUOROACETATE SALT	
91	4-AMINO-1-[2-(1-ETHOXY-ETHOXYMETHYL)-[1,3]DIOXOLAN-4-YL]-1H-PYRIMIDIN-2-ONE	
92	4-AMINO-1-[2-(1-CYCLOHEXYLOXY-ETHOXYMETHYL)-[1,3]DIOXOLAN-4-YL]-1H-PYRIMIDIN-2-ONE	

No.	Name	Structure	Chiral
93	1-(2' (S)-ETHOXYMETHYL-[1,3]DIOXOLAN-4' (S)-YL)-4-ETHYLAMINO-1H-PYRIMIDIN-2-ONE		Chiral
94	[1-(2-Hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid 2-isopropyl-5-methyl-cyclohexyl ester		
95	Carbonic acid 4-(4-amino-2-oxo-2H-[1,3]dioxolan-2-ylmethyl ester 2-isopropyl-5-methyl-cyclohexyl ester		
96	2-METHYL-HEXANOIC ACID [1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-AMIDE		Chiral
97	4-AMINO-1-[2-(1-BUTOXY-ETHOXYMETHYL)-[1,3]DIOXOLAN-4-YL]-1H-PYRIMIDIN-2-ONE		

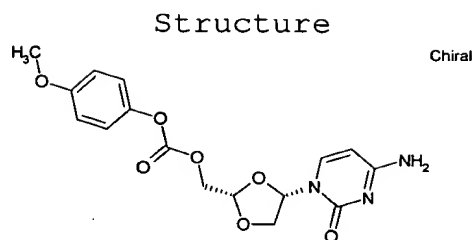
No.	Name	Structure
98	(2S,4S) 4-AMINO-1-(2-BENZYLOXYMETHYL-[1,3]DIOXOLAN-4-YL)-1H-PYRIMIDIN-2-ONE	
99	2-ETHYL-HEXANOIC ACID [1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-EXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-AMIDE	
100	2,4,6-Triisopropylbenzoic acid 4-(4-amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethyl ester	
101	ADAMANTANE-1-CARBOXYLIC ACID 4-(4-BENZYLOXYCARBONYLAMINO-2-EXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER	
102	ADAMANTANE-1-CARBOXYLIC ACID 4-{4-[(ADAMANTANE-1-CARBONYL)-AMINO]-2-EXO-2H-PYRIMIDIN-1-YL}-[1,3]DIOXOLAN-2-YLMETHYL ESTER	

No.	Name	Structure
103	CARBONIC ACID 4-[4-(4-CHLORO-PHENOXYCARBONYLAMINO)-2-OXO-2H-PYRIMIDIN-1-YL]-[1,3]DIOXOLAN-2-YLMETHYL ESTER 4-CHLORO-PHENYL ESTER	
104	[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-CARBAMIC ACID 4-CHLORO-PHENYL ESTER TRIFLUOROACETATE SALT	
105	CARBONIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER 4-CHLORO-PHENYL ESTER TRIFLUOROACETATE SALT	
106	(2S,4S)-2-(2'-(METHYLPHENYLACETOXY)METHYL-4-(CYTOSIN-1'-YL)-1,3-DIOXOLANE HYDROCHLORIDE	
107	2,2-DIMETHYLHEXANOIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-1,3-DIOXOLAN-2-YLMETHYL ESTER HYDROCHLORIDE	

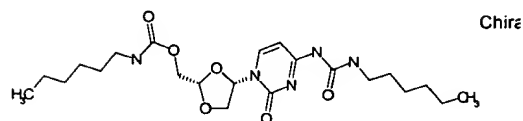
No.	Name	Structure
108	1-BENZYL-3-[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-UREA	
109	BENZYL-CARBAMIC ACID 4-[4-(3-BENZYL-UREIDO)-2-OXO-2H!-PYRIMIDIN-1-YL]-[1,3]DIOXOLAN-2-YLMETHYL ESTER	
110	ADAMANTANE-1-CARBOXYLIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER	
111	5-(BENZYL-TERT-BUTOXYCARBONYL-AMINO)-PENTANOIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER	
112	CARBONIC ACID 4(S)-(4'-AMINO-2'-OXO-2H-PYRIMIDIN-1'-YL)-[1,3]DIOXOLAN-2(S)-YLMETHYL ESTER 4-(5'',6''-DIMETHOXY-1''-OXO-INDAN-2''-YLIDENEMETHYL)-2,6-DIMETHYL-PHENYL ESTER	

No.	Name	Structure
113	4-AMINO-1-[2-(1-METHOXY-CYCLOHEXYLOXYMETHYL)-[1,3]DIOXOLAN-4-YL]-1H-PYRIMIDIN-2-ONE	
114	5-(BENZYL-TERT-BUTOXYCARBONYL-AMINO)-PENTANOIC ACID 4-{4-[5-(BENZYL-TERT-BUTOXYCARBONYL-AMINO)-PENTANOYLAMINO]-2-OXO-2H-PYRIMIDIN-1-YL}-[1,3]DIOXOLAN-2-YLMETHYL ESTER	
115	BENZYL-{4-[1-(2-HYDROXYMETHYL)-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YLCARBAMOYL]-BUTYL}-CARBAMIC ACID TERT-BUTYL ESTER	
116	CARBONIC ACID 4-(4-BENZYLOXYCARBONYLAMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER 4-METHOXY-PHENYL ESTER	
117	4-AMINO-1-{2-[1-(1,1-DIMETHYL-PROPOXY)-ETHOXYMETHYL]-[1,3]DIOXOLAN-4-YL}-1H-PYRIMIDIN-2-ONE	

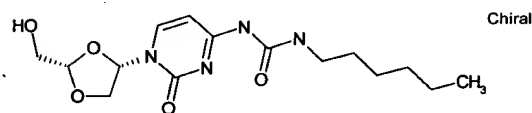
No. Name  
 118 CARBONIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER 4-METHOXY-PHENYL ESTER



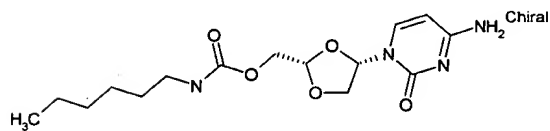
119 HEXYL-CARBAMIC ACID 4-[4-(3-HEXYL-UREIDO)-2-OXO-2H-PYRIMIDIN-1-YL]-[1,3]DIOXOLAN-2-YLMETHYL ESTER



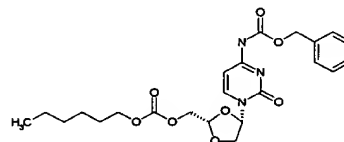
120 1-HEXYL-3-[1-(2-HYDROXYMETHYL)-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-UREA



121 HEXYL-CARBAMIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER

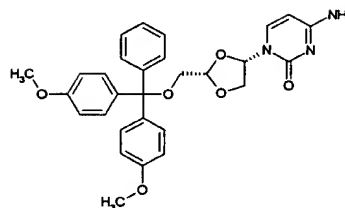


122 CARBONIC ACID 4-(4-BENZYLOXYCARBONYLAMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER HEXYL ESTER

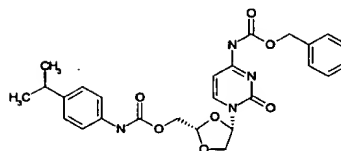


- No. Name  
 123 4-AMINO-1-{2-[BIS-(4-METHOXY-PHENYL)-PHENYL-METHOXYMETHYL]-[1,3]DIOXOLAN-4-YL}-1H-PYRIMIDIN-2-ONE

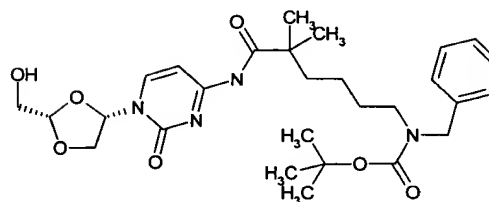
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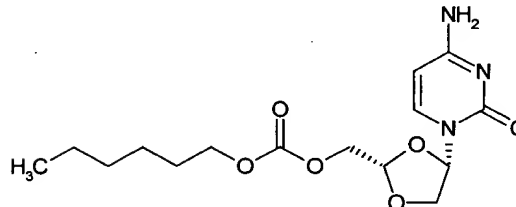
- 124 {1-[2-(4-ISOPROPYL-PHENYL-CARBAMOYLOXYMETHYL)-[1,3]DIOXOLAN-4-YL]-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL}-CARBAMIC ACID BENZYL ESTER



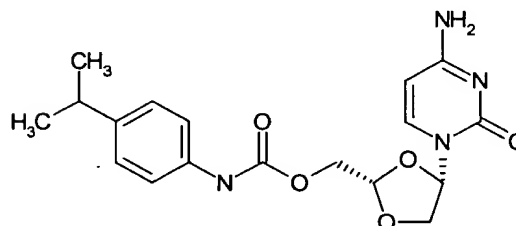
- 125 Benzyl-{5-[1-(2-hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl-carbamoyl]-5-methylhexyl}-carbamic acid tert-butyl ester



- 126 CARBONIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER HEXYL ESTER



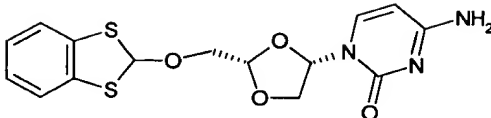
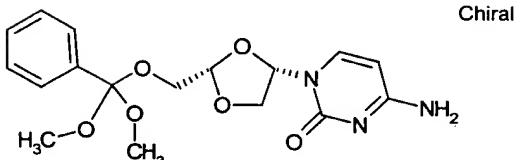
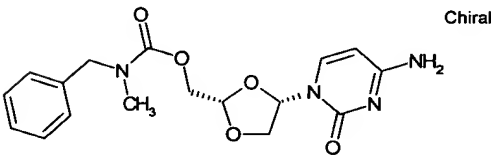
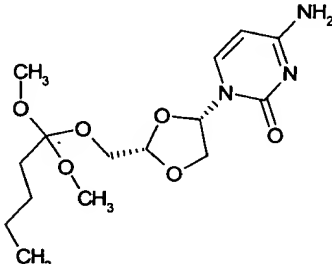
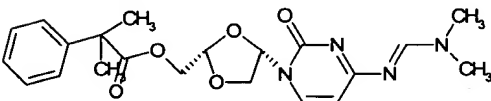
- 127 (4-ISOPROPYL-PHENYL)-CARBAMIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER





No.	Name	Structure
128	4-AMINO-1-[5-(2-METHYL-4-OXO-4#H!-BENZO[1,3]DIOXIN-2-YLOXYMETHYL)-TETRAHYDRO-FURAN-2-YL]-1#H!-PYRIMIDIN-2-ONE; COMPOUND WITH TRIFLUORO-ACETIC ACID	
129	(2S,4S)-2-(1''-ADMANTANEACETOXY)METHYL-4-(4'-N,N-DIMETHYLAMINOMETHYLENE-CYTOSIN-1'-YL)-1,3-DIOXOLANE	
130	(2S,4S)-2-(2''-DIPHENYLACETOXYMETHYL)-4-(4'-N,N-DIMETHYLAMINOMETHYLENE-CYTOSIN-1'-YL)-1,3-DIOXOLANE	
131	(2S,4S)-2-(BENZYLOXYCARBONYL-L-VALINOXYMETHYL)-4-(4'-N,N-DIMETHYLAMINOMETHYLENE-CYTOSIN-1'-YL)-1,3-DIOXOLANE	
132	6-(Benzyl-tert-butoxycarbonyl-amino)-2,2-dimethyl-hexanoic acid 4-[4-(dimethylamino-methyleneamino)-2-oxo-2H-pyrimidin-1-yl]-[1,3]dioxolan-2-ylmethyl ester	

No.	Name	Structure
133	2,2-Dimethyl-propionic acid 4-[4-(dimethylamino)-methyleneamino]-2-oxo-2H-pyrimidin-1-yl]-[1,3]dioxolan-2-ylmethyl ester	
134	4-AMINO-1-{2-[(4-METHOXY-PHENYL)-DIPHENYL-METHOXYMETHYL]-[1,3]DIOXOLAN-4-YL}-1H-PYRIMIDIN-2-ONE	
135	DIHEXYLCARBAMIC ACID 4(S)-(4'-AMINO-2'-OXO-2H-PYRIMIDIN-1'-YL)-[1,3]DIOXOLAN-2(S)-YLMETHYL ESTER	
136	4-(BENZO[1,3]DITHIOL-2-YLAMINO)-1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-1H-PYRIMIDIN-2-ONE	
137	DECYL-CARBAMIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER	

No.	Name	Structure
138	4-AMINO-1-[2-(BENZO[1,3]DITHIOL-2-YLOXYMETHYL)-[1,3]DIOXOLAN-4-YL]-1H-PYRIMIDIN-2-ONE	
139	4-AMINO-1-[2-(DIMETHOXY-PHENYL-METHOXYMETHYL)-[1,3]DIOXOLAN-4-YL]-1H-PYRIMIDIN-2-ONE	
140	BENZYL-METHYL-CARBAMIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER	
141	4-AMINO-1-[2-(1,1-DIMETHOXY-PENTYLOXYMETHYL)-[1,3]DIOXOLAN-4-YL]-1H-PYRIMIDIN-2-ONE	
142	(2S,4S)-2-(2''-DIMETHYLPHENYLACETOXY)METHYL-4-(4'-N,N-DIMETHYLAMINOMETHYLENE-CYTOSIN-1,-YL)-1,3-DIOXOLANE	

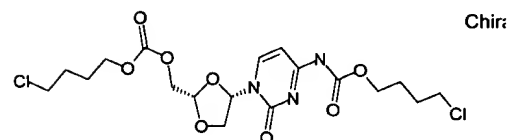
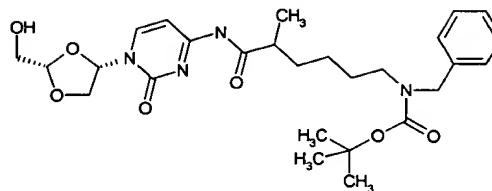
No.	Name	Structure
143	(2S,4S)-2-(4''-N,N-DIMETHYLAMINOPHENYLACETOXY)METHYL-4-(4'-N,N-DIMETHYLAMINOMETHYLENE-CYTOSIN-1'-YL)-1,3-DIOXOLANE	
144	4-(9-PHENYL-9#H!-XANTHEN-9-YLAMINO)-1-[2-(9-PHENYL-9#H!-XANTHEN-9-YLOXYMETHYL)-[1,3]DIOXOLAN-4-YL]-1#H!-PYRIMIDIN-2-ONE	
145	1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-4-(9-PHENYL-9#H!-XANTHEN-9-YLAMINO)-1#H!-PYRIMIDIN-2-ONE	
146	4-AMINO-1-[2-(9-PHENYL-9#H!-XANTHEN-9-YLOXYMETHYL)-[1,3]DIOXOLAN-4-YL]-1#H!-PYRIMIDIN-2-ONE	
147	THIOCARBONIC ACID O-[4(S)-(4'-AMINO-2'-OXO-2H-PYRIMIDIN-1'-YL)-[1,3]DIOXOLAN-2(S)-YLMETHYL] ESTER O-PHENYL ESTER	Chiral

No.	Name	Structure
148	Acetic acid 6-acetoxy-5-acetoxymethyl-2-[4-(4-benzyloxycarbonylamino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethoxy]-2-methyl-tetrahydro-[1,3]dioxolo[4,5-b]pyran-7-yl ester	
149	6-(Benzyl-tert-butoxycarbonyl-amino)-2-methyl-hexanoic acid 4-[4-(dimethylamino-methyleneamino)-2-oxo-2H-pyrimidin-1-yl]-[1,3]dioxolan-2-ylmethylester	
150	CARBONIC ACID HEXYL ESTER 4-(4-HEXYLOXYCARBONYLAMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER	
151	Acetic acid 6-acetoxy-5-acetoxymethyl-2-[4-(4-amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethoxy]-2-methyl-tetrahydro-[1,3]dioxolo[4,5-b]pyran-7-yl ester	
152	4-[(BENZOTRIAZOL-1-YLMETHYL)-AMINO]-1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-1H-PYRIMIDIN-2-ONE	

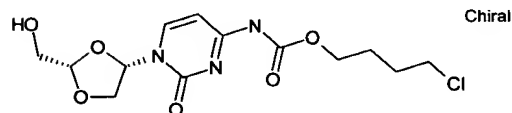
No.	Name	Structure
153	BENZOIC ACID 4-(4-BENZYLOXYCARBONYLAMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER	
154	4-AMINO-1-[2-(1-BENZYLOXY-1-METHYLETHOXYMETHYL)-[1,3]DIOXOLAN-4-YL]-1H-PYRIMIDIN-2-ONE	
155	(2S,4S)-2-[2''-(2'''-NITROPHENYL)-2''-METHYLPROPIONYLOXYMETHYL]-4-CYTOSIN-1'-YL-1,3-DIOXOLANE	
156	(2S,4S)-2-(N,N-DIMETHYL-L-VALINYLOXYMETHYL)-4-CYTOSIN-1'-YL-1,3-DIOXOLANE	
157	(2S,4S)-(3''-DIPHENYL-2''-METHYLPROPIOXYMETHYL)-4-CYTOSIN-1'-YL-1,3-DIOXOLANE	

- | No. | Name  |
|-----|---|
| 158 | Benzyl-{5-[1-(2-hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydropyrimidin-4-ylcarbamoyl]-hexyl}-carbamic acid tert-butyl ester |
| 159 | CARBONIC ACID 4-[4-(4-CHLORO-BUTOXYCARBONYLAMINO)-2-EXO-2H-PYRIMIDIN-1-YL]-[1,3]DIOXOLAN-2-YLMETHYL ESTER 4-CHLORO-BUTYL ESTER    |
| 160 | [1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-EXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-CARBAMIC ACID 4-CHLORO-BUTYL ESTER                      |
| 161 | 2,6-Dimethyl-benzoic acid 4-(4-amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethyl ester                                      |
| 162 | 1-[2-(2,6-DIMETHYL-BENZOYLOXYMETHYL)-[1,3]DIOXOLAN-4-YL]-2-EXO-1,2-DIHYDRO-PYRIMIDIN-4-YL-AMMONIUM; CHLORIDE                      |

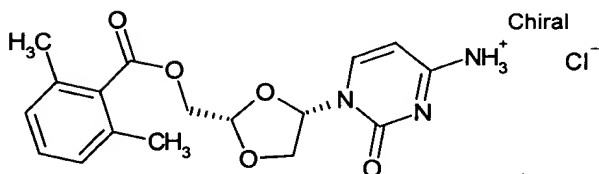
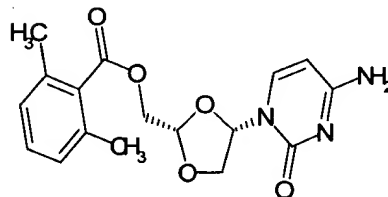
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Chiral



Chiral

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No.	Name	Structure
163	BENZOIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER	
164	CARBONIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER 3-DIMETHYLAMINO-PROPYL ESTER TRIFLUORO-ACETIC ACID SALT	
165	N-{[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YLAMINO]-METHYL}-BENZAMIDE	
166	5-(Benzyl-tert-butoxycarbonyl-amino)-2,2-dimethyl-5-oxo-pentanoic acid 4-[4-(dimethylamino-methyleneamino)-2-oxo-2H-pyrimidin-1-yl]-[1,3]dioxolan-2-ylmethyl ester	
167	[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-CARBAMIC ACID 2-BENZENESULFONYL-ETHYL ESTER	



No.	Name	Structure
168	N-[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-4-NITRO-BENZENESULFONAMIDE	
169	[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-CARBAMIC ACID 4-DIMETHYLAMINO-BUTYL ESTER TRIFLUOROACETIC ACID SALT	
170	4-AMINO-1-[2-(DIETHOXY-PHENYL-METHOXYMETHYL)-[1,3]DIOXOLAN-4-YL]-1H-PYRIMIDIN-2-ONE	
171	(S,S) 4-(DI-PROP-2'-YNYL-AMINO)-1-(2''-HYDROXYMETHYL-[1,3]DIOXOLAN-4''-YL)-1H-PYRIMIDIN-2-ONE	
172	1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-4-(PHENYLAMINOMETHYL-AMINO)-1H-PYRIMIDIN-2-ONE	

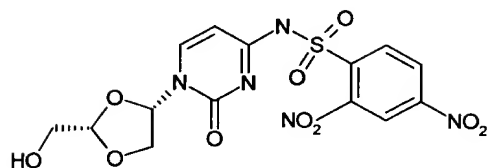
No.	Name	Structure	
173	(S,S)-4-AMINO-1-(2'-PROP-2'-YNYLOXYMETHYL-[1,3]DIOXOLAN-4'-YL)-1H-PYRIMIDIN-2-ONE		Chiral
174	4-METHOXY-BENZOIC ACID 4-[4-(4-METHOXY-BENZOYLAMINO)-2-OXO-2H-PYRIMIDIN-1-YL]-[1,3]DIOXOLAN-2-YLMETHYL ESTER		Chiral
175	N-[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-4-METHOXY-BENZAMIDE		Chiral
176	4-METHOXY-BENZOIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER		Chiral
177	4-AMINO-1-(2-TRIMETHOXYMETHOXYMETHYL-[1,3]DIOXOLAN-4-YL)-1H-PYRIMIDIN-2-ONE		Chiral

No.	Name	Structure	
178	(S,S)-4-AMINO-1-(2'-ETHOXYMETHYL-[1,3]DIOXOLAN-4'-YL)-1H-PYRIMIDIN-2-ONE		Chiral
179	(S,S)-1-(2'-ALLYLOXYMETHYL-[1,3]DIOXOLAN-4'-YL)-4-AMINO-1H-PYRIMIDIN-2-ONE		Chiral
180	(S,S)-1-(2'-ETHOXYMETHYL-[1,3]DIOXOLAN-4'-YL)-4-ETHYLAMINO-1H-PYRIMIDIN-2-ONE		Chiral
181	CARBONIC ACID 4-NITRO-BENZYL ESTER 4-[4-(4-NITRO-BENZYLOXYCARBONYLAMINO)-2-OXO-2H-PYRIMIDIN-1-YL]-[1,3]DIOXOLAN-2-YLMETHYL ESTER		Chiral
182	[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-CARBAMIC ACID 4-NITRO-BENZYL ESTER		Chiral

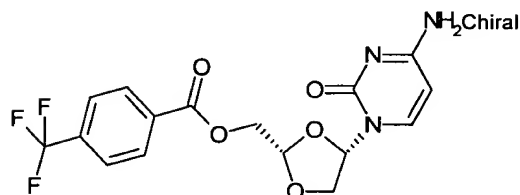
No.	Name	Structure	Chiral
183	CARBONIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER 4-NITRO-BENZYL ESTER HYDROCHLORIDE SALT		Chiral Cl <sup>-</sup>
184	3,4,6-TRI-O-BENZOYL-1,2-O-(1-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYLOXY)-BENZYL)-α-D-GLUCOPYRANOSE		
185	4-AMINO-1-{2-[TRIS-(4-METHOXY-PHENYL)-METHOXYMETHYL]-[1,3]DIOXOLAN-4-YL}-1H-PYRIMIDIN-2-ONE		
186	3,5-DI-TERT-BUTYL-BENZOIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER		Chiral
187	3,4-DICHLORO-BENZOIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER		Chiral

No. Name  
 188 N-[1-(2-HYDROXYMETHYL-  
 [1,3]DIOXOLAN-4-YL)-2-  
 OXO-1,2-DIHYDRO-  
 PYRIMIDIN-4-YL]-2,4-  
 DINITRO-  
 BENZENESULFONAMIDE

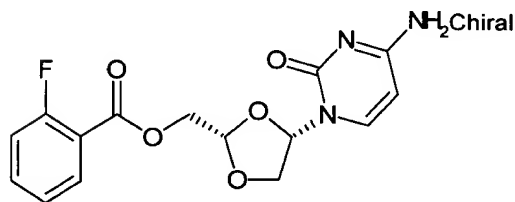
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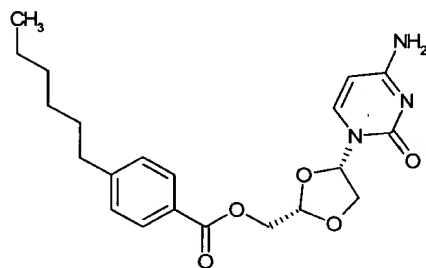
189 4-TRIFLUOROMETHYL-  
 BENZOIC ACID 4-(4-  
 AMINO-2-OXO-2H-  
 PYRIMIDIN-1-YL)-  
 [1,3]DIOXOLAN-2-YL  
 METHYL ESTER



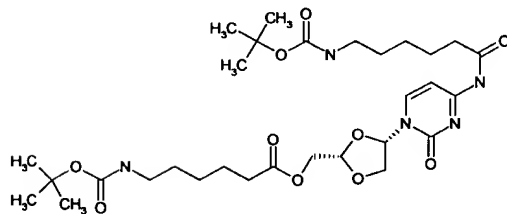
190 2-FLUORO-BENZOIC ACID 4-(4-  
 AMINO-2-OXO-2H-  
 PYRIMIDIN-1-YL)-  
 [1,3]DIOXOLAN-2-YL  
 METHYL ESTER



191 4-HEXYL-BENZOIC ACID 4-(4-  
 AMINO-2-OXO-2H-  
 PYRIMIDIN-1-YL)-  
 [1,3]DIOXOLAN-2-YL  
 METHYL ESTER



192 6-TERT!-  
 BUTOXYCARBONYLAMINO-  
 HEXANOIC ACID 4-[4-(6-  
 TERT-  
 BUTOXYCARBONYLAMINO-  
 HEXANOYLAMINO)-2-OXO-  
 2H-PYRIMIDIN-1-YL]-  
 [1,3]DIOXOLAN-2-YL  
 METHYL ESTER

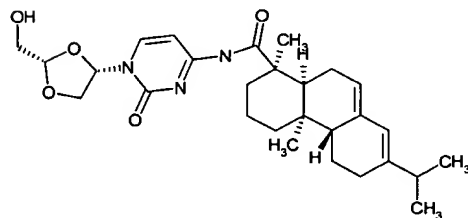
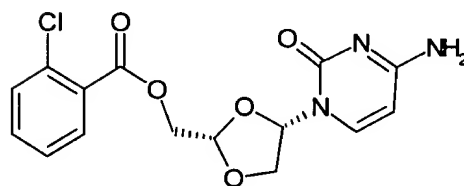
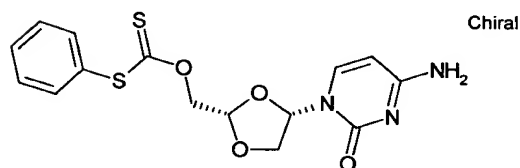
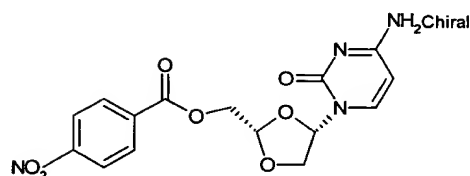
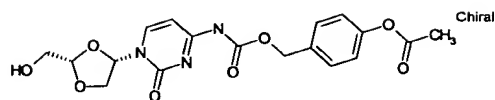


No.	Name	Structure
193	{5-[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2- OXO-1,2-DIHYDRO-PYRIMIDIN-4- YLCARBAMOYL]-PENTYL)- CARBAMIC ACID TERT- BUTYL ESTER	
194	6-TERT-BUTOXYCARBONYLAMINO- HEXANOIC ACID 4-(4- AMINO-2-OXO-2H- PYRIMIDIN-1-YL)- [1,3]DIOXOLAN-2- YLMETHYL ESTER	
195	4-AMINO-1-{2-[ DIMETHOXY-(4-METHOXY- PHENYL)-METHOXYMETHYL]- [1,3]DIOXOLAN-4-YL)- 1#H!-PYRIMIDIN-2-ONE	
196	8-PHENYL-OCTANOIC ACID 4-[2-OXO-4-(8-PHENYL- OCTANOYLAMINO)-2H- PYRIMIDIN-1-YL]- [1,3]DIOXOLAN-2-YL METHYL ESTER	
197	8-PHENYL-OCTANOIC ACID [1-(2-HYDROXYMETHYL- [1,3]DIOXOLAN-4-YL)-2- OXO-1,2-DIHYDRO- PYRIMIDIN-4-YL]-AMIDE	

No.	Name	Structure
198	8-PHENYL-OCTANOIC ACID 4-(4-AMINO-2-OXO-2H- PYRIMIDIN-1-YL)- [1,3]DIOXOLAN-2-YL METHYL ESTER	
199	4-Amino-1-(2- triethoxymethoxymethyl- [1,3]dioxolan-4-yl)-1H- pyrimidin-2-one	 Chiral
200	4-AMINO-1-[2- (DIMETHOXY-#P!-TOLYL- METHOXYMETHYL)- [1,3]DIOXOLAN-4-YL]- 1#H!-PYRIMIDIN-2-ONE	
201	3-[4-(4-AMINO-2-OXO-2H- PYRIMIDIN-1-YL)- [1,3]DIOXOLAN-2-YL METHOXY]-ACRYLIC ACID ETHYL ESTER	
202	ACETIC ACID 4-{1-[2-(4- ACETOXY- BENZYLOXYCARBONYLOXYMET HYL)-[1,3]DIOXOLAN-4- YL]-2-OXO-1,2-DIHYDRO- PYRIMIDIN-4-YL CARBAMOYLOXYMETHYL}- PHENYL ESTER	 Chiral

- | No. | Name  |
|-----|---|
| 203 | ACETIC ACID 4-[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-EXO-1,2-DIHYDRO-PYRIMIDIN-4-YLCARBAMOYLOXYMETHYL]-PHENYL ESTER  |
| 204 | 4-NITRO-BENZOIC ACID 4-(4-AMINO-2-EXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YL METHYL ESTER  |
| 205 | DITHIOCARBONIC ACID O-[4-(4-AMINO-2-EXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YL METHYL] ESTER S-PHENYL ESTER  |
| 206 | 2-CHLORO-BENZOIC ACID 4-(4-AMINO-2-EXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YL METHYL ESTER   |
| 207 | 7-ISOPROPYL-2,4A-DIMETHYL-1,2,3,4,4A,4B,5,6,10,10A-DECAHYDRO-PHENANTHRENE-2-CARBOXYLIC ACID [1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-EXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-AMIDE |

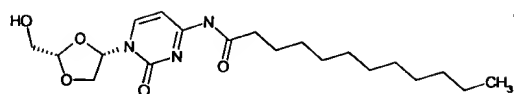
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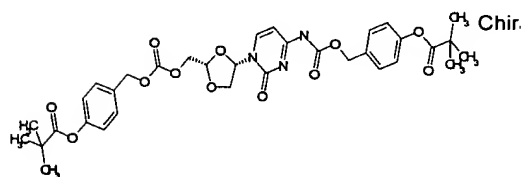
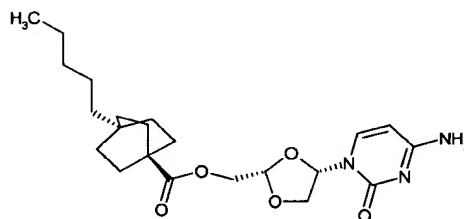
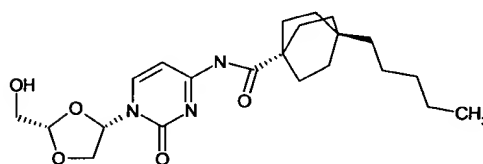
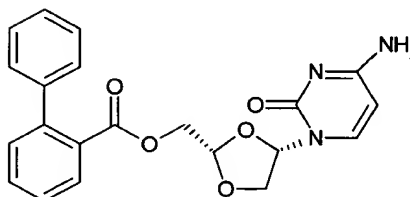


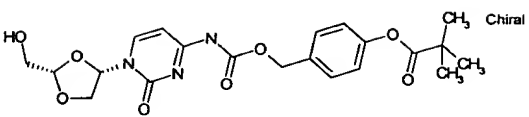
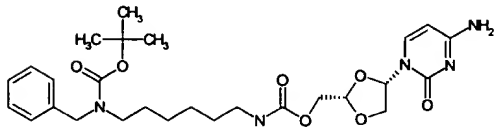
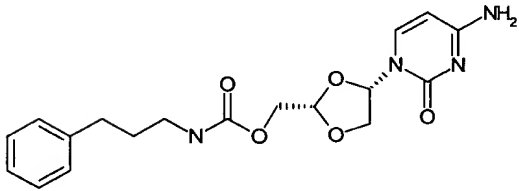

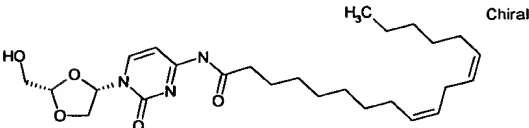
- | No. | Name  |
|-----|---|
| 208 | DODECANOIC ACID [1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-EXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-AMIDE   |
| 209 | BIPHENYL-2-CARBOXYLIC ACID 4-(4-AMINO-2-EXO-2#H!-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YL METHYL ESTER  |
| 210 | 4-PENTYL-BICYCLO[2.2.2]OCTANE-1-CARBOXYLIC ACID [1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-EXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-AMIDE   |
| 211 | 4-PENTYL-BICYCLO[2.2.2]OCTANE-1-CARBOXYLIC ACID 4-(4-AMINO-2-EXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YL METHYL ESTER   |
| 212 | 2,2-DIMETHYL-PROPIONIC ACID 4-(1-{2-[4-(2,2-DIMETHYL-PROPIONYLOXY)-BENZYLOXYCARBONYLOXYMETHYL]-[1,3]DIOXOLAN-4-YL}-2-EXO-1,2-DIHYDRO-PYRIMIDIN-4-YLCARBAMOYLOXYMETHYL)-PHENYL ESTER |

## Structure



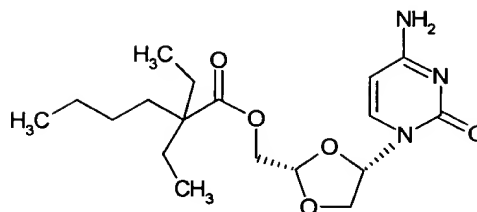
Chiral



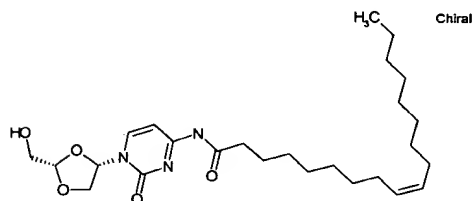
No.	Name	Structure
213	2,2-DIMETHYL-PROPIONIC ACID 4-[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-EXO-1,2-DIHYDRO-PYRIMIDIN-4-YLCARBAMOYLOXYMETHYL]-PHENYL ESTER	
214	{ 6-[4-(4-AMINO-2-EXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHOXYCARBONYLAMINO]-HEXYL}-BENZYL-CARBAMIC ACID TERT-BUTYL ESTER	
215	(3-PHENYL-PROPYL)-CARBAMIC ACID 4-(4-AMINO-2-EXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER	
216	Octadec-9-enoic acid [1-(2-hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-amide	
217	OCTADECA-9,12-DIENOIC ACID [1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-EXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-AMIDE	

No. Name  
 218 2,2-DIETHYL-HEXANOIC  
 ACID 4-(4-AMINO-2-OXO-  
 2H-PYRIMIDIN-1-YL)-  
 [1,3]DIOXOLAN-2-YL  
 METHYL ESTER

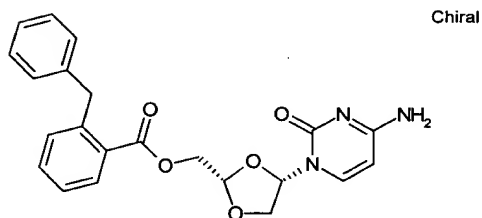
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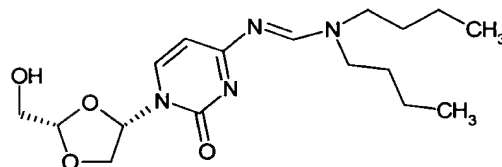
219 OCTADEC-9-ENOIC ACID  
 [1-(2-HYDROXYMETHYL-  
 [1,3]DIOXOLAN-4-YL)-2-  
 OXO-1,2-DIHYDRO-  
 PYRIMIDIN-4-YL]-AMIDE



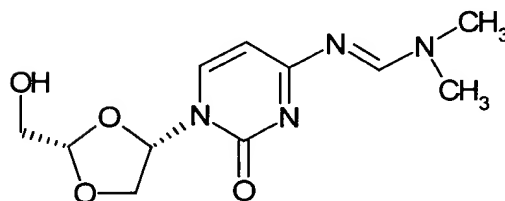
220 BIPHENYL-2-CARBOXYLIC  
 ACID 4-(4-AMINO-2-OXO-  
 2H-PYRIMIDIN-1-YL)-  
 [1,3]DIOXOLAN-2-YL  
 METHYL ESTER



221 N,N-Dibutyl-N'-[1-(2-  
 hydroxymethyl-  
 [1,3]dioxolan-4-yl)-2-  
 oxo-1,2-dihydro-  
 pyrimidin-4-yl]-  
 formamidine



222 N'-[1-(2-HYDROXYMETHYL-  
 [1,3]DIOXOLAN-4-YL)-2-  
 OXO-1,2-DIHYDRO-  
 PYRIMIDIN-4-YL]-N,N-  
 DIMETHYL-FORMAMIDINE

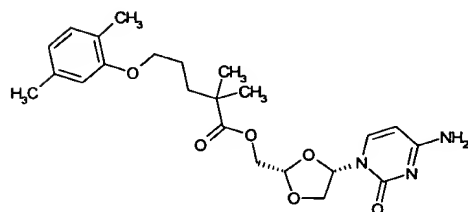
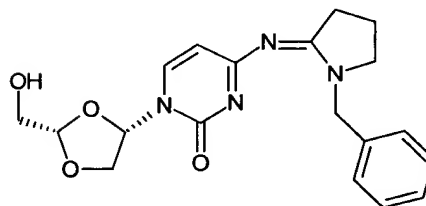
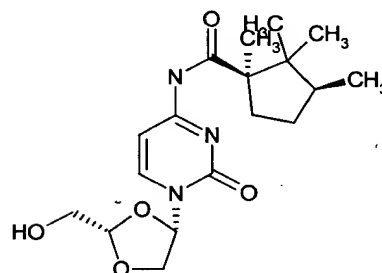
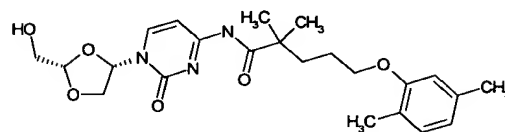
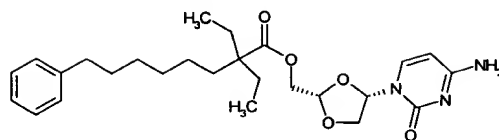


No.	Name	Structure
223	1-PHENYL-CYCLOPROPANECARBOXYLIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YL METHYL ESTER	
224	2-METHYL-2-(2-NITRO-PHENYL)-PROPIONIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER HYDROCHLORIDE SALT	
225	1-PHENYL-CYCLOHEXANECARBOXYLIC ACID [1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-AMIDE	
226	1-PHENYL-CYCLOHEXANECARBOXYLIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YL METHYL ESTER	
227	2,2-DIMETHYL-8-PHENYL-OCTANOIC ACID [1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-AMIDE	

No.	Name	Structure
228	N' - [1 - (2-HYDROXYMETHYL- [1,3]DIOXOLAN-4-YL) -2- OXO-1,2-DIHYDRO- PYRIMIDIN-4-YL] -N,N- DIMETHYL-ACETAMIDINE	
229	1-PHENYL- CYCLOPENTANECARBOXYLIC ACID [1-(2- HYDROXYMETHYL- [1,3]DIOXOLAN-4-YL) -2- OXO-1,2-DIHYDRO- PYRIMIDIN-4-YL] -AMIDE	
230	N' - [1 - (2-HYDROXYMETHYL- [1,3]DIOXOLAN-4-YL) -2- OXO-1,2-DIHYDRO- PYRIMIDIN-4-YL] -N,N- DIISOPROPYL-FORMAMIDINE	
231	HEXAHYDRO-2,5-METHANO- PENTALENE-3A-CARBOXYLIC ACID [1-(2- HYDROXYMETHYL- [1,3]DIOXOLAN-4-YL) -2- OXO-1,2-DIHYDRO- PYRIMIDIN-4-YL] -AMIDE	
232	HEXAHYDRO-2,5-METHANO- PENTALENE-3A-CARBOXYLIC ACID 4-(4-AMINO-2-OXO- 2H-PYRIMIDIN-1-YL) - [1,3]DIOXOLAN-2-YL METHYL ESTER	

- | No. | Name   |
|-----|--|
| 233 | 2,2-DIETHYL-8-PHENYL-OCTANOIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YL METHYL ESTER                               |
| 234 | 5-(2,5-DIMETHYL-PHENOXY)-2,2-DIMETHYL-PENTANOIC ACID [1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-AMIDE |
| 235 | 1,2,2,3-TETRAMETHYL-CYCLOPENTANECARBOXYLIC ACID [1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-AMIDE      |
| 236 | 4-(1-BENZYL-PYRROLIDIN-2-YLIDENEAMINO)-1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-1H-PYRIMIDIN-2-ONE                                     |
| 237 | 4-AMINO-1-{2-[4-(2,5-DIMETHYL-PHENOXY)-1,1-DIMETHYL-BUTOXYMETHYL]-[1,3]DIOXOLAN-4-YL}-1H-PYRIMIDIN-2-ONE                             |

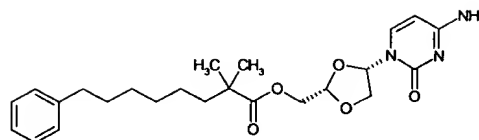
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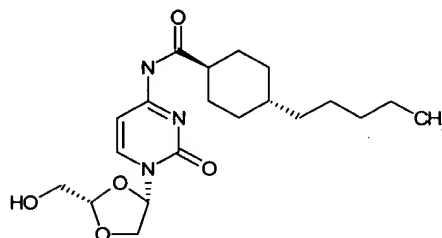
Chiral

No. Name  
 238 2,2-DIMETHYL-8-PHENYL-  
 OCTANOIC ACID 4-(4-  
 AMINO-2-OXO-2H-  
 PYRIMIDIN-1-YL)-  
 [1,3]DIOXOLAN-2-YL  
 METHYL ESTER

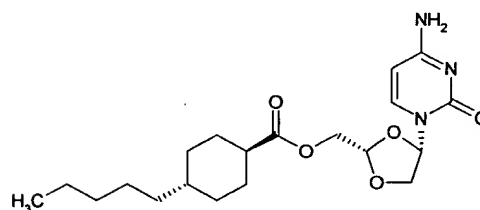
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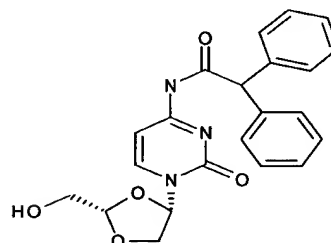
239 4-PENTYL-  
 CYCLOHEXANECARBOXYLIC  
 ACID [1-(2-  
 HYDROXYMETHYL-  
 [1,3]DIOXOLAN-4-YL)-2-  
 OXO-1,2-DIHYDRO-  
 PYRIMIDIN-4-YL]-AMIDE



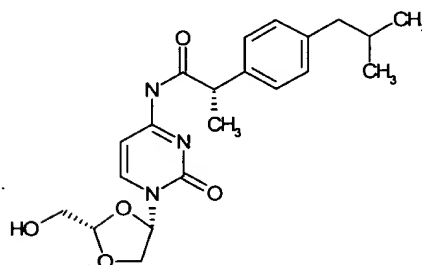
240 4-PENTYL-  
 CYCLOHEXANECARBOXYLIC  
 ACID 4-(4-AMINO-2-OXO-  
 2H-PYRIMIDIN-1-YL)-  
 [1,3]DIOXOLAN-2-YL  
 METHYL ESTER



241 N-[1-(2-HYDROXYMETHYL-  
 [1,3]DIOXOLAN-4-YL)-2-  
 OXO-1,2-DIHYDRO-  
 PYRIMIDIN-4-YL]-2,2-  
 DIPHENYL-ACETAMIDE

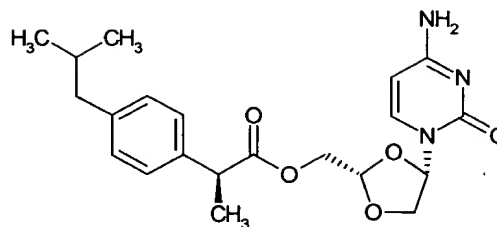


242 N-[1-(2-HYDROXYMETHYL-  
 [1,3]DIOXOLAN-4-YL)-2-  
 OXO-1,2-DIHYDRO-  
 PYRIMIDIN-4-YL]-2-(4-  
 ISOBUTYL-PHENYL)-  
 PROPIONAMIDE

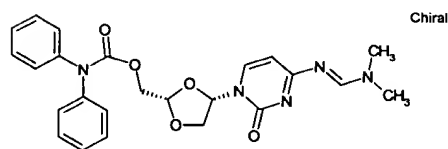


No. Name  
 243 2-(4-ISOBUTYL-PHENYL)-  
 PROPIONIC ACID 4-(4-  
 AMINO-2-OXO-2H-  
 PYRIMIDIN-1-YL)-  
 [1,3]DIOXOLAN-2-YL  
 METHYL ESTER

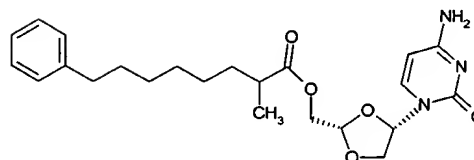
Structure



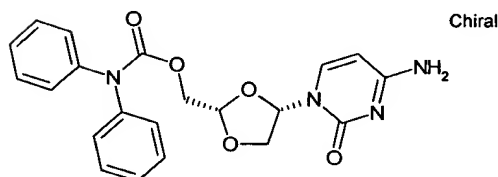
244 DIPHENYL-CARBAMIC ACID  
 4-[4-(DIMETHYLAMINO-  
 METHYLENEAMINO)-2-OXO-  
 2H-PYRIMIDIN-1-YL]-  
 [1,3]DIOXOLAN-2-YL  
 METHYL ESTER



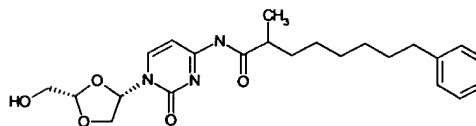
245 2-METHYL-8-PHENYL-  
 OCTANOIC ACID 4-(4-  
 AMINO-2-OXO-2H-  
 PYRIMIDIN-1-YL)-  
 [1,3]DIOXOLAN-2-YL  
 METHYL ESTER



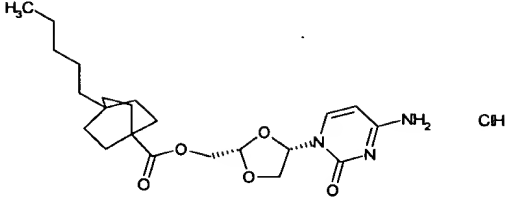
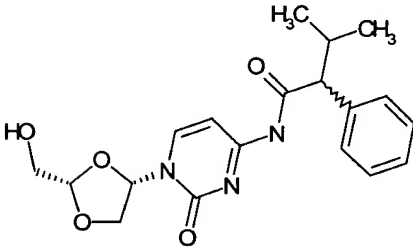
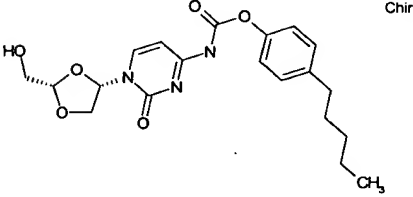
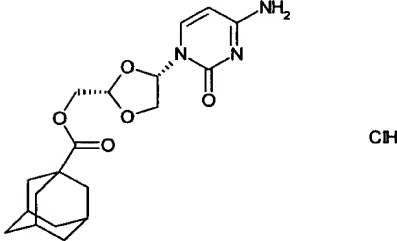
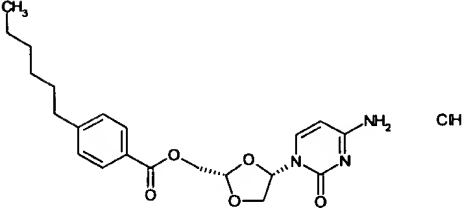
246 DIPHENYL-CARBAMIC ACID  
 4-(4-AMINO-2-OXO-2H-  
 PYRIMIDIN-1-YL)-  
 [1,3]DIOXOLAN-2-YL  
 METHYL ESTER



247 2-Methyl-8-phenyl-  
 octanoic acid [1-(2-  
 hydroxymethyl-  
 [1,3]dioxolan-4-yl)-2-  
 oxo-1,2-dihydro-  
 pyrimidin-4-yl]-amide

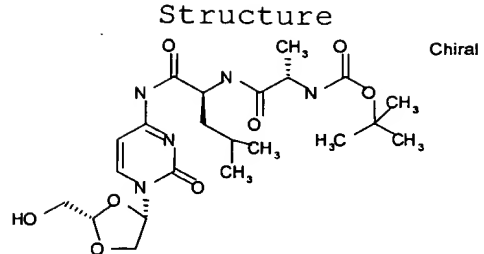
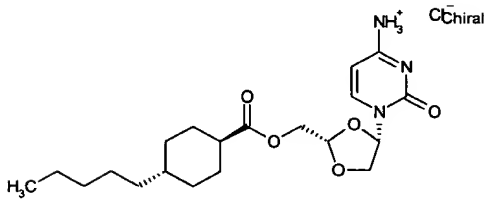
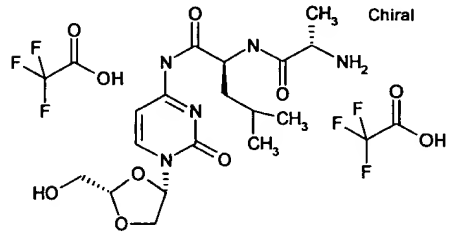
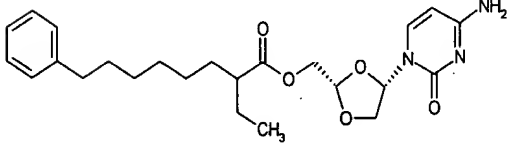
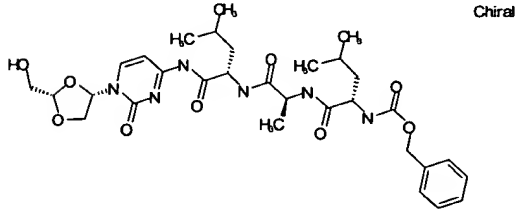




No.	Name	Structure
248	4-PENTYL-BICYCLO[2.2.2]OCTANE-1-CARBOXYLIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER; HYDROCHLORIDE SALT	
249	#N!-[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-3-METHYL-2-PHENYL-BUTYRAMIDE	
250	[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-CARBAMIC ACID 4-PENTYL-PHENYL ESTER	
251	Adamantane-1-carboxylic acid 4-(4-amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-yl methyl ester	
252	4-HEXYL-BENZOIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YL METHYL ESTER; HYDROCHLORIDE SALT	

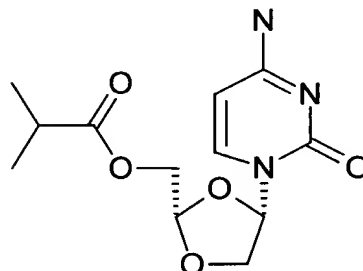
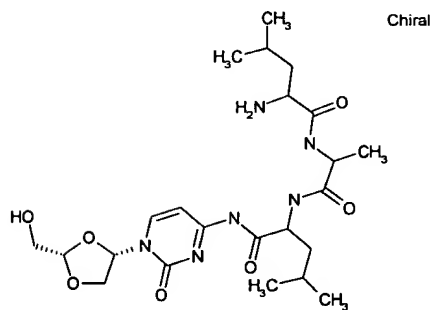
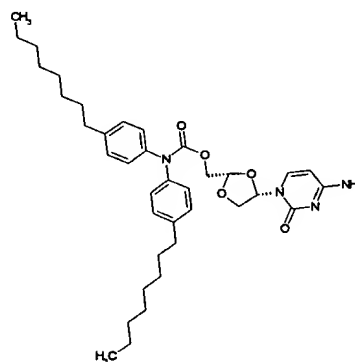
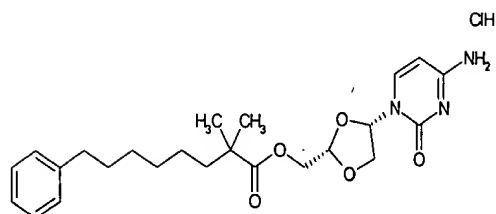
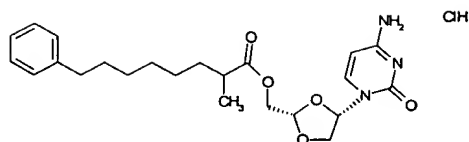
No.	Name	Structure
253	2-OXO-1-[2-(1-PHENYL-CYCLOHEXANECARBOXYLOXYMETHYL)-[1,3]DIOXOLAN-4-YL]-1,2-DIHYDRO-PYRIMIDIN-4-YL-AMMONIUM; CHLORIDE	
254	{1-[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL-CARBAMOYL]-3-METHYLBUTYL}-CARBAMIC ACID BENZYL ESTER	
255	[4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YL METHOXY]-PHOSPHONO-ACETATE BIS-AMMONIUM SALT	
256	2-tert-Butyl-8-phenyloctanoic acid 4-(4-amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-yl methyl ester	
257	2-AMINO-4-METHYLPENTANOIC ACID [1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-AMIDE	

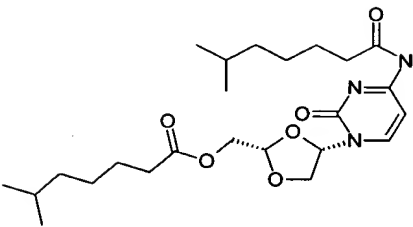
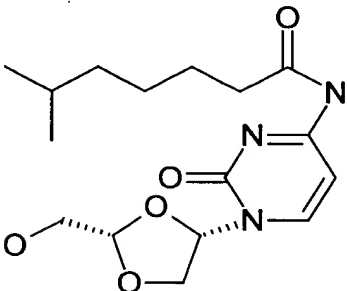
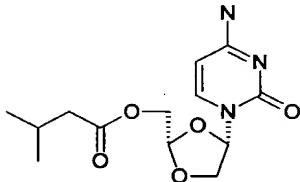
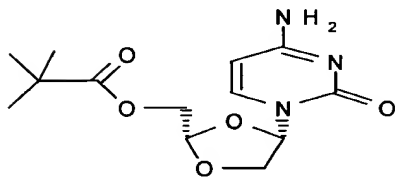
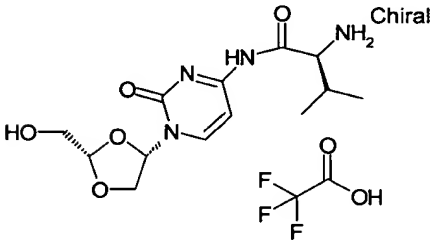
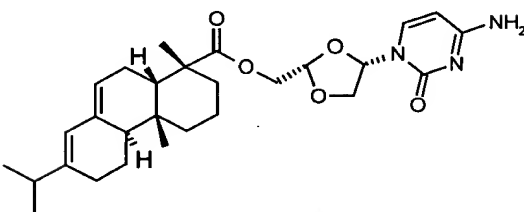
No.	Name	Structure
258	BENZOIC ACID 4-(4-ACETYLAMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER	
259	BENZOIC ACID 4-(4-ACETYLAMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER	
260	1-(2-[2-(4-ISOBUTYL-PHENYL)-PROPIONYLOXYMETHYL]-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YLAMMONIUM; CHLORIDE	
261	8-Phenyl-octanoic acid 4-(4-amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethyl ester hydrochloride	
262	3-METHYL-2-PHENYL-BUTYRIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER	

- | No. | Name  | Structure  |
|-----|---|--|
| 263 | (1-{1-[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YLCARBAMOYL]-3-METHYLBUTYLCARBAMOYL}-ETHYL)-CARBAMIC ACID TERT-BUTYL ESTER                        |    |
| 264 | 2-OXO-1-[2-(4-PENTYLCYCLOHEXANECARBONYLOXYMETHYL)-[1,3]DIOXOLAN-4-YL]-1,2-DIHYDRO-PYRIMIDIN-4-YL-AMMONIUM CHLORIDE  |    |
| 265 | 2-(2-AMINO-PROPIONYLAMINO)-4-METHYL-PENTANOIC ACID [1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-AMIDE, BIS TRIFLUOROACETIC ACID SALT             |   |
| 266 | 2-ETHYL-8-PHENYLOCTANOIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER  |  |
| 267 | [1-(1-{1-[1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YLCARBAMOYL]-3-METHYLBUTYLCARBAMOYL}-ETHYLCARBAMOYL)-3-METHYLBUTYL]-CARBAMIC ACID BENZYL ESTER |  |

- | No. | Name   |
|-----|--|
| 268 | 2-METHYL-8-PHENYL-OCTANOIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER HYDROCHLORIDE   |
| 269 | 2,2-DIMETHYL-8-PHENYL-OCTANOIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER HYDROCHLORIDE   |
| 270 | BIS-(4-OCTYL-PHENYL)-CARBAMIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YLMETHYL ESTER  |
| 272 | 2-AMINO-4-METHYL-PENTANOIC ACID (1-{1-[1-(2-HYDROXYMETHYL)-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL CARBAMOYL]-3-METHYL-BUTYLCARBAMOYL}-ETHYL)-AMIDE |
| 275 | ISOBUTYRIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL)-[1,3]DIOXOLAN-2-YL METHYL ESTER  |

## Structure

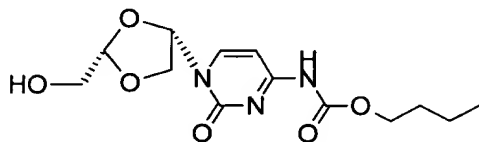


No.	Name	Structure
276	6-METHYL-HEPTANOIC ACID 4-[4-(6-METHYL- HEPTANOYLAMINO)-2-OXO- 2H-PYRIMIDIN-1-YL]- [1,3]DIOXOLAN-2-YL METHYL ESTER	
277	6-METHYL-HEPTANOIC ACID [1-(2-HYDROXYMETHYL- [1,3]DIOXOLAN-4-YL)-2- OXO-1,2-DIHYDRO- PYRIMIDIN-4-YL]-AMIDE	
278	3-METHYL-BUTYRIC ACID 4-(4-AMINO-2-OXO-2H- PYRIMIDIN-1-YL)- [1,3]DIOXOLAN-2-YL METHYL ESTER	
279	2,2-DIMETHYL-PROPIONIC ACID 4-(4-AMINO-2-OXO- 2H-PYRIMIDIN-1-YL)- [1,3]DIOXOLAN-2-YL METHYL ESTER	
280	2-Amino-N-[1-(2- hydroxymethyl- [1,3]dioxolan-4-yl)-2- oxo-1,2-dihydro- pyrimidin-4-yl]-3- methyl-butamide; trifluoroacetic acid salt	
281	7-ISOPROPYL-2,4A- DIMETHYL- 1,2,3,4,4A,4B,5,6,10,10 A-DECAHYDRO- PHENANTHRENE-2- CARBOXYLIC ACID [1-(2- HYDROXYMETHYL- [1,3]DIOXOLAN-4-YL)-2- OXO-1,2-DIHYDRO- PYRIMIDIN-4-YL]-ESTER	

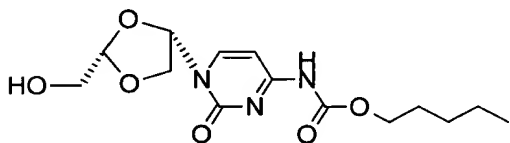
The following are examples of additional compounds in accordance with the invention:

5

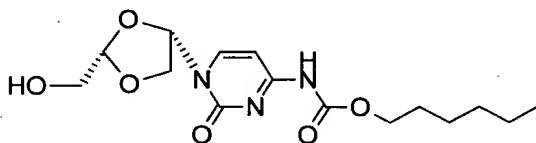
[1-(2-Hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-carbamic acid butyl ester



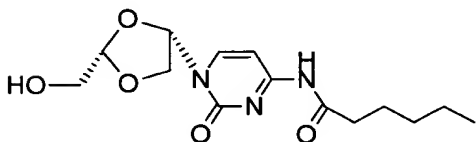
[1-(2-Hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-carbamic acid pentyl ester



10 [1-(2-Hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-carbamic acid hexyl ester

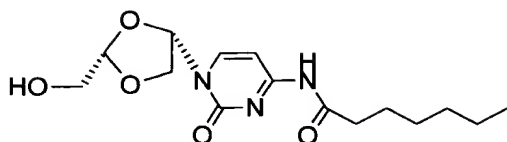


Hexanoic acid [1-(2-hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-amide

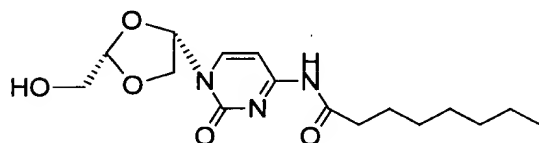


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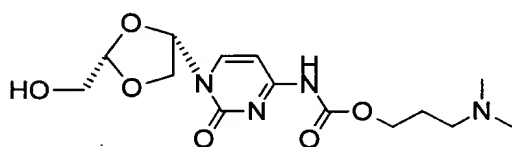
Heptanoic acid [1-(2-hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-amide



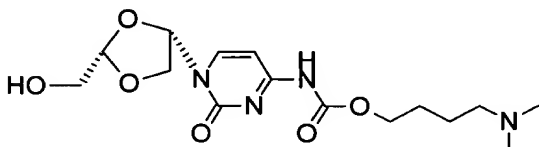
- 5 Octanoic acid [1-(2-hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-amide



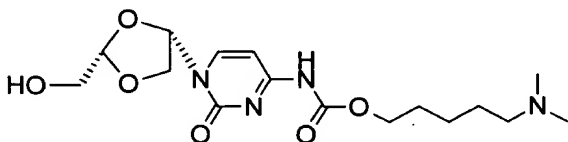
- 10 [1-(2-Hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-carbamic acid 3-dimethylamino-propyl ester



- [1-(2-Hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-carbamic acid 4-dimethylamino-butyl ester

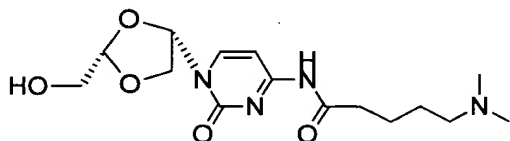


- 15 [1-(2-Hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-carbamic acid 5-dimethylamino-pentyl ester

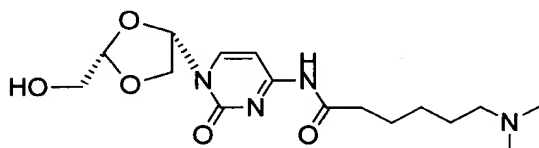




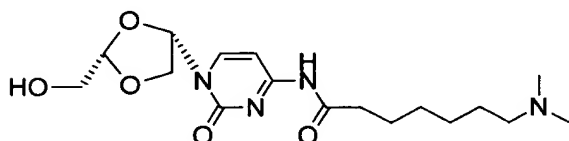
- 5 5-Dimethylamino-pentanoic acid [1-(2-hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-amide



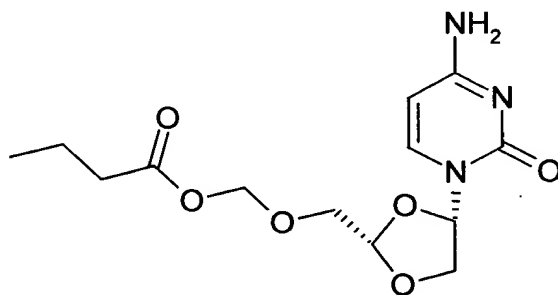
- 6-Dimethylamino-hexanoic acid [1-(2-hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-amide
- 10 amide



- 7-Dimethylamino-heptanoic acid [1-(2-hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-amide

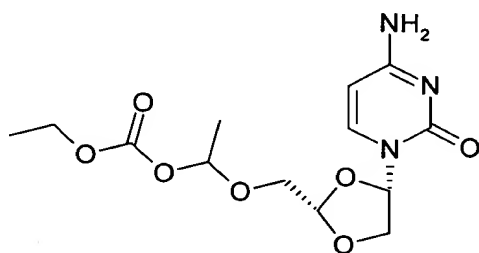


- 15 Acetic acid 4-(4-amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethoxymethyl ester

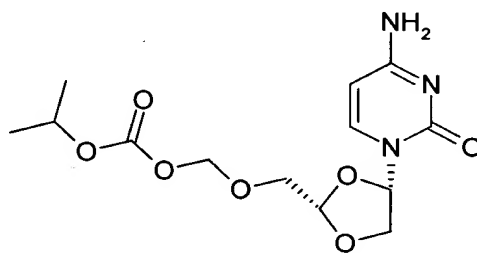


Butyric acid 4-(4-amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethoxymethyl ester

5



Carbonic acid 1-[4-(4-amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethoxy]-ethyl ester ethyl ester



Carbonic acid 4-(4-amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethoxymethyl ester isopropyl ester

10 (2S, 4S) N-[1-(2-Hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-2-piperidin-4-yl-acetamide trifluoroacetate salt

15 (2S, 4S) Piperidin-4-yl-acetic acid 4-(4-amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethyl ester trifluoroacetate salt

20 (2S, 4S) 2-Amino-3-methyl-butyric acid 4-(4-amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethyl ester trifluoroacetate salt

(2S, 4S) 2-Amino-N-[1-(2-hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-3-methyl-butyramide trifluoroacetate salt

25

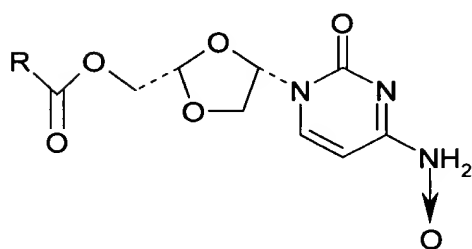
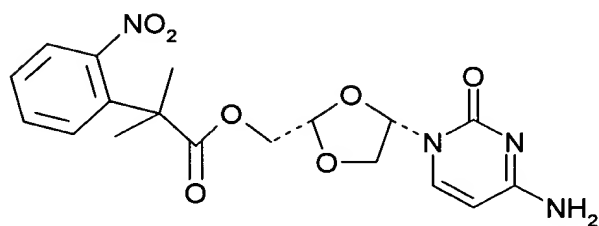
(2S, 4S) 4-Amino-1-[2-(tetrahydro-pyran-2-yloxymethyl)-[1,3]dioxolan-4-yl]-1H-pyrimidin-2-one

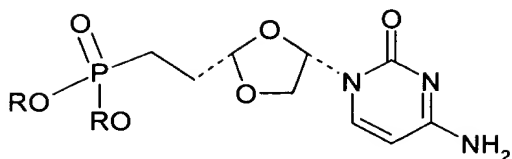
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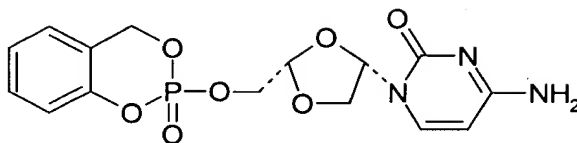


Further examples are:

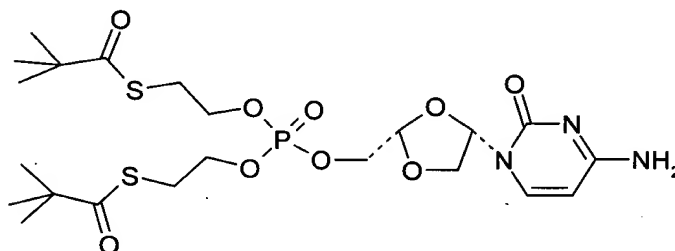




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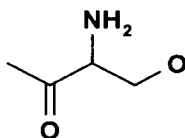
The compounds of formula (I) have a cis geometrical configuration. Moreover, the compounds of formula (I) exhibit the 'unnatural' nucleoside configuration, that is they are L-enantiomers. Preferably, the compounds of formula (I) are provided substantially free of the corresponding D-enantiomers, that is to say no more than about 5% w/w of the corresponding D-nucleoside, preferably no more than about 2% w/w, in particular less than about 1% w/w is present.

The compounds formula (I) include compounds in which the hydrogen of the 2-hydroxymethyl group and/or one or both of the hydrogens of a base amino group(s) is replaced by alkyl, alkenyl, aryl, a heteroaromatic group or a nonaromatic ring group, or are replaced by  $-C(O)R^6$  or -

- 5 C(O)OR<sup>6</sup> groups in which R<sup>6</sup> is alkyl, alkenyl, aryl optionally substituted by alkyl, a heteroaromatic group optionally substituted by alkyl, or a nonaromatic ring group.
- 10 With regard to the compounds of formula (I), unless otherwise specified, any alkyl or alkenyl moiety present advantageously contains up to 24 carbon atoms, particularly 4 to 18 carbon atoms. Any aryl moiety present preferably contains 6 to 24 carbon atoms, for
- 15 example, phenyl, naphthyl, and biphenyl groups.
- In the compounds of formula (I), R<sup>1</sup>, R<sup>3</sup> and/or R<sup>4</sup> can also exhibit an amino acid radical or an amino acid chain. Unless specified otherwise, the term "amino acid" used
- 20 herein includes naturally-occurring amino acids as well as non natural analogs as those commonly used by those skilled in the art of chemical synthesis and peptide chemistry. A list of non natural amino acids may be found in "The Peptides", vol. 5, 1983, Academic Press, Chapter 6
- 25 by D.C. Roberts and F. Vellaccio. Example of naturally occurring amino acid includes alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), cysteine (Cys), glutamine (Gln), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu),
- 30 lysine (Lys), methionine (Met), phenylalanine (Phe), ornithine (Orn), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr), and valine (Val).

5 Preferably, the amino acid radical or amino acid chain exhibits at least one amino acid radical selected from Ala, Glu, Val, Leu, Ile, Pro, Phe, Tyr or Typ.

By the term "amino acid residue" and "amino acid chain  
10 residue" is meant an amino acid or amino acid chain preferably lacking the carboxy terminal hydroxyl group. For example, the amino acid residue of serine is preferably:



15 Pharmaceutically acceptable salts of the compounds of formula (I) include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acids include hydrochloric, hydrobromic, sulphuric, nitric, perchloric, fumaric,  
20 maleic, phosphoric, glycollic, lactic, salicylic, succinic, toleune-p-sulphonic, tartaric, acetic, citric, methanesulphonic, formic, benzoic, malonic, naphthalene-2-sulphonic and benzenesulphonic acids. Other  
25 acids such as oxalic, while not in themselves pharmaceutically acceptable, may be useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts.

5 Salts derived from appropriate bases include alkali metal (e.g. sodium), alkaline earth metal (e.g. magnesium), ammonium and  $\text{NR}_4^+$  (where R is  $\text{C}_{1-4}$  alkyl) salts.

The compounds of the invention either themselves possess  
10 anticancer activity and/or are metabolizable to such compounds.

By the term "amino acid chain" is meant two or more, preferably 2 to 6, amino acid residues covalently bound  
15 via a peptide or thiopeptide bond.

The alkyl groups, including alkylene structures, can be straight chain or branched. In addition, within the alkyl or alkylene groups, one or more  $\text{CH}_2$  can be replaced, in  
20 each case independently, by  $-\text{O}-$ ,  $-\text{CO}-$ ,  $-\text{S}-$ ,  $-\text{SO}_2-$ ,  $-\text{NH}-$ ,  $-\text{N}(\text{C}_{1-4}\text{-alkyl})-$ ,  $-\text{N}(\text{C}_{6-10}\text{-aryl})-$ ,  $-\text{CS}-$ ,  $-\text{C}=\text{NH}-$ , or  $-\text{N}(\text{CO}-\text{O}-\text{C}_{1-4}\text{-alkyl})-$ , in manner in which O atoms are not directly bonded to one another. In addition, one or more  $-\text{CH}_2\text{CH}_2-$  can be replaced, in each case independently, by  $-\text{CH}=\text{CH}-$  or  
25  $-\text{C}=\text{C}-$ . Further, alkyl and alkenyl groups can be optionally substituted by halogen, e.g., Cl and F.

Aryl can be unsubstituted or optionally substituted by one or more of  $\text{NO}_2$ ,  $\text{C}_{1-8}\text{-alkyl}$ ,  $\text{C}_{1-8}\text{-alkoxy}$ ,  $-\text{COOH}$ ,  $-\text{CO}-\text{O}-\text{C}_{1-8}\text{-alkyl}$  and halo (e.g. Cl and F) groups.  
30

- 5 The non-aromatic C<sub>3-20</sub> groups, which optionally contain 1-3 heteroatoms, are unsubstituted or optionally substituted by one or more of C<sub>1-8</sub>-alkyl, C<sub>1-8</sub>-alkoxy, OH, C<sub>1-8</sub>-hydroxyalkyl, and -CO-O-C<sub>1-8</sub>-alkyl groups.
- 10 By the term "heteroaromatic" is meant an unsaturated ring structure containing 5 to 10 ring atoms wherein 1 to 3 ring atoms are each selected from N, O and S. Examples of heteroaromatic groups include but are not limited to:
- 15 furyl, thiophenyl, pyrrolyl, imidazolyl, pyrazoyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyridyl, pyrimidinyl, triazolyl, tetrazolyl, oxadrazolyl, thiadiazolyl, thiopyranyl, pyrazinyl, benzofuryl, benzothiophenyl, indolyl, benzimidazolyl, benzopyrazolyl, benzoxazolyl, benzisoxazolyl, benzothiozolyl,
- 20 benzisothiazolyl, benzoxadiazolyl, quinolinyl, isoquinolinyl, carbazolyl, acridinyl, cinnolinyl and quinazolinyl.

Nonaromatic ring groups preferably contain 3-20 ring atoms

25 in which 1-3 ring atoms are in each case selected from N, O and S. Preferred nonaromatic ring groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, piperazinyl, piperidinyl, morpholinyl, thiomorpholinyl, pyrrolidinyl, adamantyl or quinuclidinyl.



5 The compounds of formula (I) include ester compounds. Such esters can be obtained by, for example, esterification of the 2-hydroxymethyl groups with a fatty acid. Typically fatty acids contain 4-22 carbon atoms. Examples of ester compounds of formula (I) include  
10 compounds in which at least one of R<sub>1</sub>, R<sub>3</sub> or R<sub>4</sub> is acetyl, propionyl, butyryl, valeryl, caprioic, caprylic, capric, lauric, myristic, palmitic, stearic, oleic, linoleic, or linolenic.

15 There is thus provided as a further aspect of the invention, methods for treating solid tumors. A further aspect of the invention, is a method of treating liver cancer or metastasis thereof, lung cancer, renal cancer, colon cancer, pancreatic cancer, uterine cancer, ovarian  
20 cancer, breast cancer, bladder cancer, melanoma and lymphoma.

Compounds of the invention can be tested for use against cancers using any of a variety of art-recognized *in vitro*  
25 models [e.g., inhibition of proliferation of cell lines such as tumor cell lines, as described herein and, for example, in Bowlin et al. (1998). *Proc. Am. Assn. for Cancer Res.* 39, #4147] or animal models [e.g., leukemic (Gourdeau et al. (2000). *Cancer Chemotherapy and*  
30 *Pharmacology*) or solid tumor (Grove et al. (1997). *Cancer Res.* 57: 3008-3011; Kadhim et al. (1997). *Cancer Res.* 57:

5 4803-4810; Rabbani et al. (1998). *Cancer Res.*58: 3461;  
Weitman et al. (2000). *Clinical Cancer Res.*6: 1574-1578)]  
xenograft animal models. See, also, USP 5,817,667.  
Clinical tests of safety (absence of toxicity) and efficacy  
are carried out and evaluated using conventional testing  
10 methods.

Nucleosides can enter cells by any of a variety of  
mechanisms. As used herein, the term "nucleoside" means a  
nucleoside, nucleoside analog, modified nucleoside, or the  
15 like, for example any of the nucleoside "prodrugs"  
described above. Mechanisms of nucleoside uptake include,  
e.g., uptake by nucleoside or nucleobase transporter  
proteins (NT), including sodium-independent, bidirectional  
equilibrative transporters such as, e.g., the *es* or *ei*  
20 transporters; by sodium-dependent, inwardly directed  
concentrative transporters such as, e.g., *cit*, *cib*, *cif*,  
*csg*, and *cs*; by nucleobase transporters; or by passive  
diffusion. For a discussion of the properties of some  
NTs, see, e.g., Mackey et al. (1981). *Cancer Research* 58,  
25 4349-4357 and Mackey et al. (1998). *Drug Resistance*  
*Updates* 1, 310-324, which are incorporated in their  
entirety by reference herein.

Methods (tests) for determining the mechanism(s) by which  
30 a nucleoside enters a cell are conventional in the art.  
Some such methods are described, e.g., in Gourdeau et al.

5 (2000). "Troxacitabine has an Unusual Pattern of Cellular Uptake and Metabolism that Results in Differential Chemosensitivity to Cytosine-Containing Nucleosides in Solid-Tumor and Leukemic Cell Lines" (submitted for publication and attached hereto as an appendix) and

10 Paterson et al. (1991) "Plasma membrane transport of nucleosides, nucleobases and nucleotides: an overview," in Imai & Nakazawa, eds., Role of adenosine and adenosine nucleotides in the biological system, Elsevier Science Publishers, which are incorporated in their entirety by

15 reference herein. Typical methods include, for example: 1) NT inhibitor studies: measuring the ability of a nucleoside of interest to inhibit proliferation of cells, e.g., cancer (malignant) cells, or measuring the uptake of a labeled nucleoside of interest into a cell, wherein the

20 nucleoside is administered to the cell in the presence or absence of one or more inhibitors of nucleoside transporters. Such inhibitors include, e.g., NBMPR (nitrobenzylmercaptapurine), which is specific for the es transporter; dipyridamole, which is specific for the es

25 and the ei NTs; and dilazep, which is specific for the NTs encoded by the genes hCNT1 and hCNT2, respectively. Reduction of activity or of uptake of a nucleoside of interest by an inhibitor of a particular NT implicates that NT in the mechanism of entry of the nucleoside into

30 the cell; whereas the absence of such a reduction suggests

5 that the NT is not involved. Methods to perform such assays are conventional and are disclosed, e.g., in Mackey et al., *supra* and in Examples 1-4.

2) Competition studies: measuring the kinetics of uptake  
10 of a labeled nucleoside which is known to be transported by a particular NT in the presence or absence of a large molar excess (e.g., about a 100 to 1000-fold excess) of an unlabeled nucleoside of interest. If the nucleoside of interest competes with the labeled nucleoside for the NT,  
15 thereby reducing or abolishing the amount of uptake of the labeled nucleoside, this implicates that NT in the mechanism of uptake of the nucleoside of interest. By contrast, the lack of such competition suggests that the NT is not involved in the uptake of the nucleoside of  
20 interest. See, e.g., Example 31 (hCNT3 experiment). Cell proliferation studies such as those described above can also be studied by comparable competition assays.

3) Competition with uridine: measuring the kinetics of uptake of a labeled nucleoside of interest in the presence  
25 of a large molar excess (e.g., about 100 to 1000-fold) of unlabeled uridine. Uridine is generally regarded as a "universal permeant," which can be taken up by cells by all of the reported human NTs. If a large excess of uridine does not inhibit the uptake of a nucleoside of  
30 interest, this indicates that the nucleoside is not

5 transported by at least any of the currently known  
nucleoside transporters and, therefore, this is consistent  
with entry into the cell by passive diffusion.

4) Competition with the nucleoside of interest, itself:  
10 measuring the kinetics of uptake of a labeled nucleoside  
of interest in the presence or absence of a large molar  
excess (e.g., about 100 to 1000-fold) of that nucleoside,  
itself, in unlabeled form. Reduction of the amount of  
labeled nucleoside taken up by a cell when excess  
15 unlabeled nucleoside is present suggests that a molecule  
with affinity for the nucleoside (e.g., a nucleoside  
transporter) participates in the uptake mechanism. By  
contrast, unchanged or increased transport of the labeled  
nucleoside indicates that the mechanism of uptake is by  
20 passive diffusion. See, e.g., Example 30 (HeLa cells; DU  
145 cells), which demonstrates that uptake of  $^3\text{H}$ -  
troxacitabine is not inhibited by a large excess of  
unlabeled troxacitabine, indicating that the mechanism of  
uptake of troxacitabine in these cells is passive  
25 diffusion.

Any of the preceding tests can be carried out with any of  
a variety of cells which express a defined number of well-  
characterized nucleoside or nucleobase transporters. In  
30 addition to cell lines which naturally express defined

5 numbers of NTs, mutant cell lines have been isolated which  
are deficient in one or more NTs, and/or one or more NTs  
can be introduced into a cell by conventional genetic  
recombinant methods. Genes encoding many NTs have been  
cloned (see, e.g., Griffiths et al. (1997) *Nat. Med.* 3:  
10 89-93; Crawford et al. (1998) *J. Biol. Chem.* 273: 5288-  
5293; Griffiths et al. (1997) *Biochem. J.* 328: 739-743;  
Ritzel et al. (1997) *Am. J. Physiol.* 272: C707-C714; Wang  
et al. (1997) *Am. J. Physiol* 273: F1058-F1065) or can be  
cloned by conventional methods; and methods of subcloning  
15 these genes into appropriate expression vectors are  
conventional. See, e.g., Sambrook, J. et al. (1989).  
*Molecular Cloning, a Laboratory Manual*. Cold Spring  
Harbor Laboratory Press, Cold Spring Harbor, NY for  
methods of cloning, subcloning, and expressing genes. A  
20 typical example of a panel of cell lines expressing  
different combinations of NTs is disclosed, e.g., in  
Mackey et al., *supra*.

5) Studies with artificial membranes, e.g., reconstituted  
25 proteoliposomes comprising known NTs: measuring the  
kinetics of uptake of a labeled nucleoside of interest,  
e.g., in the presence or absence of inhibitors. See,  
e.g., Mackey et al., *supra*.

5 It will be further appreciated that the amount of a  
compound of the invention required for use in treatment  
will vary not only with the particular compound selected  
but also with the route of administration, the nature of  
the condition being treated and the age and condition of  
10 the patient and will be ultimately at the discretion of  
the attendant physician or veterinarian.

In a preferred dosage regimen (regime, schedule), the  
compound a nucleoside analog of the invention) is  
15 administered to a patient at least daily for a period of  
about 2 to 10 consecutive days, preferably for about 3 to  
7, more preferably for about 4 to 6, most preferably for  
about 5 days. This treatment is repeated, for example,  
every 2 to 5 weeks, preferably every 3 to 4 weeks,  
20 particularly about every 4 weeks.

The amount of nucleoside analog to be administered using  
the above dosage regimen can be determined by conventional,  
routine procedures, e.g., administering increasing amounts  
25 of the compound in order to determine the maximum tolerated  
dose.

For troxacitabine administration to a patient having a  
solid tumor, a preferred dosage range is about 1.2 to about  
30 1.8 mg/m<sup>2</sup>/day, more preferably about 1.5 mg/m<sup>2</sup>/day.  
Sufficient time is allowed for the patient to recover from

5 this treatment (e.g., for the patient to recover an  
adequate white blood count to withstand another round of  
therapy). Generally the time for recovery is about 2-5  
weeks. After the recovery period, another round of daily  
doses is administered as above. A compound of the  
10 invention is preferably administered daily as described  
above about every 2 to 5 weeks, more preferably about every  
3 to 4 or every 3 to 5 weeks. This dosage regimen can be  
repeated as necessary.

15 For troxacitabine administration to a patient having  
leukemia, higher amounts of the drug can be tolerated. The  
preferred dosage range for troxacitabine for this  
indication is about 3 to about 8 mg/m<sup>2</sup>/day, preferably about  
5 to about 8 mg/m<sup>2</sup>/day, and most preferably about 8  
20 mg/m<sup>2</sup>/day. For treatment of leukemia, only one cycle of  
administration is generally required, although additional  
cycles can be administered, provided that the drug does not  
reach toxic levels.

25 Optimal dosages for any of the nucleoside analogs of the  
invention can be determined without undue experimentation.  
Using the daily dosage regimen (schedule) described above,  
one of skill in the art can routinely determine, using  
conventional methods, the maximum tolerable dosage for any  
30 of the nucleosides described herein. Optimal dosages will  
vary, of course, with parameters such as age, weight and



5 physical condition of the patient, nature and stage of the  
disease, stability and formulation of the compound, route  
of administration, or the like. In general, because  
nucleosides modified with lipophilic substituents undergo  
more efficient passive diffusion through cell membranes  
10 than does troxicitabine, the dosages used for these  
nucleoside analogs can be lower than those for  
troxacitabine, for example, 10 to 100 fold lower.

Compounds of the invention can be administered, using the  
15 dosage regimens and dosage amounts discussed above, to any  
patient having cancer who would benefit from the treatment.  
For example, the patient to be treated can exhibit cancer  
cells that are resistant to one or more of other, commonly  
administered, anticancer drugs, e.g., gemcitabine or ara-C  
20 (cytarabine). In another aspect, the malignant cells are  
deficient in nucleoside membrane transport via nucleoside  
or nucleobase transporter proteins, e.g., they lack or  
comprise mutant forms of known nucleoside transporters such  
as, for example, *es*, *ei*, *cit*, *cib*, *cif*, *csg*, and *cs*. In  
25 another aspect, the drug (compound) enters the cancer cell  
predominantly (e.g., at least about 50%) by passive  
diffusion.

While it is possible that, for use in therapy, a compound  
30 of the invention may be administered as the raw chemical

5 it is preferable to present the active ingredient as a pharmaceutical formulation.

The invention thus further provides a pharmaceutical composition comprising a compound of formula (I) or a  
10 pharmaceutically acceptable salt thereof together with one or more pharmaceutically acceptable carriers therefor and, optionally, other therapeutic and/or prophylactic ingredients. The carrier(s) must be 'acceptable' in the sense of being compatible with the other ingredients of  
15 the formulation and not deleterious to the recipient thereof.

Pharmaceutical formulations include those suitable for oral, rectal, nasal, topical (including buccal and  
20 sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration or in a form suitable for administration by inhalation or insufflation. The formulations may, where appropriate, be conveniently presented in discrete dosage  
25 units and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association the active compound with liquid carriers or finely divided solid carriers or both and then, if necessary, shaping the product into the desired  
30 formulation.

5     Pharmaceutical formulations suitable for oral  
administration may conveniently be presented as discrete  
units such as capsules, cachets or tablets each containing  
a predetermined amount of the active ingredient; as a  
powder or granules; as a solution, a suspension or as an  
10    emulsion. The active ingredient may also be presented as a  
bolus, electuary or paste. Tablets and capsules for oral  
administration may contain conventional excipients such as  
binding agents, fillers, lubricants, disintegrants, or  
wetting agents. The tablets may be coated according to  
15    methods well known in the art. Oral liquid preparations  
may be in the form of, for example, aqueous or oily  
suspensions, solutions, emulsions, syrups or elixirs, or  
may be presented as a dry product for reconstitution with  
water or other suitable vehicle before use. Such liquid  
20    preparations may contain conventional additives such as  
suspending agents, emulsifying agents, non-aqueous vehicles  
(which may include edible oils), or preservatives.

The compounds according to the invention may also be  
25    formulated for parenteral administration (e.g. by  
injection, for example bolus injection or continuous  
infusion) and may be presented in unit dose form in  
ampoules, pre-filled syringes, small volume infusion or in  
multi-dose containers with an added preservative. The  
30    compositions may take such forms as suspensions,  
solutions, or emulsions in oily or aqueous vehicles, and  
may contain formulatory agents such as suspending,

5 stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution, for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use.

10

For topical administration to the epidermis the compounds according to the invention may be formulated as ointments, creams or lotions, or as a transdermal patch. Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilising agents, dispersing agents, suspending agents, thickening agents, or coloring agents.

20

Formulations suitable for topical administration in the mouth include lozenges comprising active ingredient in a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

25

30 Pharmaceutical formulations suitable for rectal administration wherein the carrier is a solid are most

5 preferably presented as unit dose suppositories. Suitable  
carriers include cocoa butter and other materials commonly  
used in the art, and the suppositories may be conveniently  
formed by admixture of the active compound with the  
softened or melted carrier(s) followed by chilling and  
10 shaping in moulds.

Formulations suitable for vaginal administration may be  
presented as pessaries, tampons, creams, gels, pastes,  
foams or sprays containing in addition to the active  
15 ingredient such carriers as are known in the art to be  
appropriate.

For intra-nasal administration the compounds of the  
invention may be used as a liquid spray or dispersible  
20 powder or in the form of drops.

Drops may be formulated with an aqueous or non-aqueous  
base also comprising one more more dispersing agents,  
solubilising agents or suspending agents. Liquid sprays  
25 are conveniently delivered from pressurised packs.

For administration by inhalation the compounds according  
to the invention are conveniently delivered from an  
insufflator, nebuliser or a pressurised pack or other  
30 convenient means of delivering an aerosol spray.

Pressurised packs may comprise a suitable propellant such

5 as dichlorodifluoromethane, trichlorofluoromethane,  
dichlorotetrafluoroethane, carbon dioxide or other  
suitable gas. In the case of a pressurised aerosol the  
dosage unit may be determined by providing a valve to  
deliver a metered amount.

10

Alternatively, for administration by inhalation or  
insufflation, the compounds according to the invention may  
take the form of a dry powder composition, for example a  
powder mix of the compound and a suitable powder base such  
15 as lactose or starch. The powder composition may be  
presented in unit dosage form in, for example, capsules or  
cartridges or e.g. gelatin or blister packs from which the  
powder may be administered with the aid of an inhalator or  
insufflator.

20

When desired the above described formulations adapted to  
give sustained release of the active ingredient may be  
employed.

25 The pharmaceutical compositions according to the invention  
may also contain other active ingredients such as  
antimicrobial agents, or preservatives.

The compounds of the invention may also be used in  
30 combination with each other and/or with other therapeutic

5 agents. In particular the compounds of the invention may be employed together with known anticancer agents.

The invention thus provides, in a further aspect, a combination comprising a compound of formula (I) or a  
10 physiologically acceptable salt thereof together with another therapeutically active agent, in particular an anticancer agent.

The combinations referred to above may conveniently be  
15 presented for use in the form of a pharmaceutical formulation and thus pharmaceutical formulations comprising a combination as defined above together with a pharmaceutically acceptable carrier therefor comprise a further aspect of the invention.  
20 Suitable therapeutic agents for use in such combinations include:

1) Alkylating agents such as:

- 2-haloalkylamines (e.g. melphalan and  
25 chlorambucil),
- 2-haloalkylsulfides,
- N-alkyl-N-nitrosoureas (e.g. carmustine, lomustine  
or  
semustine),
- 30 • aryltriazines (e.g. decarbazine),
- mitomycins (e.g. mitomycin C),

- 5
  - methylhydrazines (e.g. procarbazine),
  - bifunctional alkylating agents (e.g. mechlorethamine),
  - carbinolamines (e.g. sibiromycin),
  - streptozotocins and chlorozotocins,
- 10
  - phosphoramidate mustards (e.g. cyclophosphamide),
  - urethane and hydantoin mustards,
  - busulfan,
  - oncovin;

2) Antimetabolites such as:

- 15
  - mercaptopurines (e.g. 6-thioguanine and 6-[methylthio]purine),
  - nucleoside (e.g.  $\beta$ -L-dioxolane cytidine),
  - azapyrimidines and pyrimidines,
  - hydroxyureas,
- 20
  - 5-fluorouracil,
  - folic acid antagonists (e.g. amethopterin),
  - cytarabines,
  - prednisones,
  - diglycoaldehydes,
- 25
  - methotrexate, and
  - cytosine rabinoside;

3) Intercalators such as:

- bleomycins and related glycoproteins,



- 5       • anthracyclines       (e.g. doxorubicin, daunorubicin, epirubicin, esorubicin, idarubicin, aclacinomycin A),
- acridines (e.g. m-AMSA),
- hycanthones,
- 10      • ellipticines (e.g. 9-hydroxyellipticine),
- actinomycins (e.g. actinocin),
- anthraquinones (e.g. 1,4-bis[(aminoalkyl)-
- amino]-9,10-anthracenediones),
- 15      • anthracene derivatives (e.g. pseudourea and bisanthrene),
- phleomycins,
- aureolic acids (e.g. mithramycin and olivomycin),
- and
- Camptothecins (e.g. topotecan);

20

#### 4) Mitotic inhibitors such as:

- dimeric catharanthus alkaloids
- vincristine, vinblastine and vindesine),
- 25      • colchicine derivatives (e.g. trimethylcolchicinic acid)
- epipodophyllotoxins and podophyllotoxins
- etoposide and teniposide),
- maytansinoids (e.g. maytansine and colubrinol),

- 5       • terpenes (e.g. helenalin, triptolide and taxol),
- steroids (e.g. 4 $\beta$ -hydroxywithanolide E),
- quassinoids (e.g. bruceantin),
- pipobroman, and
- methylglyoxals (e.g.
- 10       methylglyoxalbis-(thiosemicarbazone);
- 5) Hormones (e.g. estrogens, androgens, tamoxifen,  
nafoxidine, progesterone, glucocorticoids, mitotane,  
prolactin);
- 15       6) Immunostimulants such as:
- human interferons, cytokines, levamisole and  
      tilorane;
- 20       7) Monoclonal and polyclonal antibodies;
- 8) Radiosensitizing and radioprotecting compounds such as:
- metronidazole and misonidazole;
- 9) Other miscellaneous cytotoxic agents such as:
- 25       • camptothecins,
- quinolinequinones,
- streptonigrin and isopropylidene azastreptonigrin),
- cisplatin, cisrhodium and related platinum series  
      complexes,

- 5       • tricothecenes (e.g. trichodermol or vermicarin A),  
          and  
      • cephalotoxines (e.g. harringtonine);

10) Enzymes, such as

- 10       • L-asparaginase;

11) Drug-resistance reversal compounds such as

P-glycoprotein inhibitors, for example Verapamil,  
cyclosporin-c, and fujimycin;

12) Cytotoxic cells such as lymphokine activated killer

- 15       -cells or T-cells;

13) Other Immunostimulants such as interleukin factors or  
antigens;

14) Polynucleotides of sense or antisensing nature;

15) Polynucleotides capable of forming triple helices with

- 20       DNA or RNA;

16) Polyethers;

17) Distamycin and analogs;

18) Taxanes such as taxol and taxotere; and

19) Agents that are protective against drug induced

- 25       toxicities such as granulocyte macrophage colony  
stimulating factor (GM-CSF) and granulocyte colony  
stimulating factor (G-CSF).

The above list of possible therapeutic agents is not  
30       intended to limit this invention in any way.

5 The individual components of such combinations may be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations.

When a compound of formula (I), or a pharmaceutically  
10 acceptable salt thereof is used in combination with a second therapeutic agent the dose of each compound may be either the same as or differ from that when the compound is used alone. Appropriate doses will be readily appreciated by those skilled in the art.

15 The compounds of formula (I) and their pharmaceutically acceptable salts may be prepared by any method known in the art for the preparation of compounds of analogous structure, for example as described in international  
20 application No PCT/CA92/00211 published under No Wo 92/20669 which is herein incorporated by reference.

Certain intermediates useful in the synthesis of the compounds of the present invention can be synthesized as  
25 generally described in J.Med.Chem. 1994, 37, 1501-1507, Lyttle et al.

It will be appreciated by those skilled in the art that for certain of the methods the desired stereochemistry of  
30 the compounds of formula (I) may be obtained either by commencing with an optically pure starting material or by resolving the racemic mixture at any convenient stage in

5 the synthesis. In the case of all the processes the  
optically pure desired product may be obtained by  
resolution of the end product of each reaction.  
It is also possible to resolve the final compound using  
chiral HPLC (high pressure liquid chromatography) as it is  
10 well known in the art.

#### **Brief Description of the Drawings**

Various other features and attendant advantages of the  
present invention will be more fully appreciated as the same  
15 becomes better understood when considered in conjunction with  
the accompanying figures, wherein:

Fig. 1 Comparative uptake of 30  $\mu\text{M}$  [ $^3\text{H}$ ]-troxacitabine in CEM  
(Panel A) and CEM/ARAC8C (Panel B) cells. [ $^3\text{H}$ ]-Uridine  
20 uptake in either the presence or absence of the hENT1  
inhibitor, NBMPR or 5 mM non-radioactive uridine was included  
for comparison as a control substrate. Each data point  
represents the mean ( $\pm$  standard deviation) of three  
determinations.

25

Fig. 2 Comparative uptake of 10  $\mu\text{M}$  [ $^3\text{H}$ ]troxacitabine (0-  
240 min) (Panel B) and 10  $\mu\text{M}$  [ $^3\text{H}$ ]D-uridine (0-6 min) (Panel  
A) in the presence ( $\blacktriangle$ ) or absence ( $\square$ ) of the hENT1  
inhibitor, 100 nM NBMPR, in DU145 cells. Each data point

5 represents the mean ( $\pm$  standard deviation) of three determinations.

Fig. 3 Comparative uptake of 10  $\mu$ M [ $^3$ H]troxacitabine and 10  $\mu$ M [ $^3$ H]D-uridine in HeLa cells. A. Uptake of  
10 [ $^3$ H]troxacitabine ( $\square$ ) and [ $^3$ H]D-uridine ( $\square$ ) in the presence of the hENT1 inhibitor, 100 nM NBMPR using a scale of 0-1500 pmol/ $10^6$  cells. B. Uptake of [ $^3$ H]troxacitabine either in the absence ( $\square$ ) or presence of 100 nM NBMPR ( $\blacktriangle$ ), 100  $\mu$ M dilazep ( $\blacktriangledown$ ), 1 mM non-radioactive troxacitabine ( $\blacklozenge$ ) or 20  
15  $\mu$ M dipyridamole ( $\bullet$ ), using an expanded scale of 0-15 pmol/ $10^6$  cells. Each data point represents the mean ( $\pm$  standard deviation) of three determinations.

Fig. 4 Comparative uptake of 10  $\mu$ M [ $^3$ H]troxacitabine and 10  $\mu$ M [ $^3$ H]D-uridine in HeLa cells transiently transfected with  
20 recombinant pcDNA3 containing either the coding sequence for: (A) hCNT1 or (B) hCNT2. Transport assays were conducted in the presence of the equilibrative transport inhibitor, 100  $\mu$ M dilazep and either in the presence ( $\square$ ) or absence ( $\blacktriangle$ ) of with the empty vector control plasmid  
25 ( $\blacktriangledown$ ). sodium, and compared to HeLa cells transiently transfected with the empty vector control plasmic ( $\blacktriangledown$ ).

Without further elaboration, it is believed that one skilled in the art can, using the preceding description,  
30 utilize the present invention to its fullest extent. The

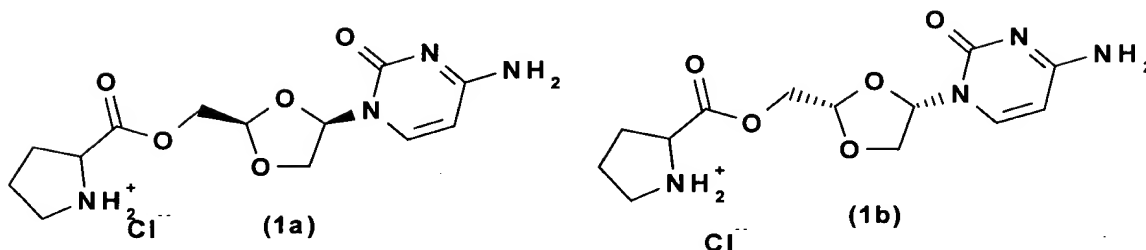
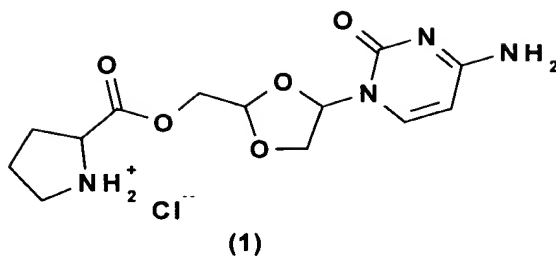
5 following preferred specific embodiments are, therefore,  
to be construed as merely illustrative, and not limitative  
of the remainder of the disclosure in any way whatsoever.  
In the foregoing and in the following examples, all  
temperatures are set forth uncorrected in degrees Celsius;  
10 and, unless otherwise indicated, all parts and percentages  
are by weight.

The entire disclosures of all applications, patents and  
publications, cited above and below, including provisional  
15 applications Serial Nos. 60,239,885 (filed October 13,  
2000) and 60/288,424 (filed May 4, 2001), are hereby  
incorporated by reference.

20

**EXAMPLE 1**

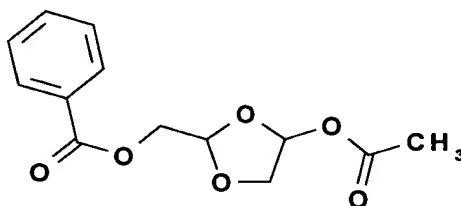
**Preparation of 2-(prolyloxymethyl)-4-cytosin-1''-yl-1,3-  
dioxolane hydrochloride ( 1, 1a, and 1b)**



5

### STEP 1

#### Preparation of 4-Acetoxy-2-(O-Benzoyloxymethyl)-dioxolane



10

A mixture of Benzyl-1,2-Dihydroxy Butyrate (116 mg; 0.97 mmol), Benzoyloxybenzaldehyde (159mg; 0.97 mmol) and *p*-toluene sulfonic acid (9mg; 0.047 mmol) in dry benzene (25ml) under argon is heated at reflux for 4 h. Solvent is then removed under reduced pressure and the remaining solid is worked-up by washing with 5% sodium bicarbonate. A purification of the crude material by chromatography on silica gel gives the expected benzyl ester. The resulting compound is dissolved in ethanol

15

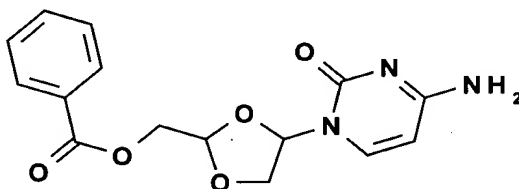
20



- 5 (25ml) and treated with Pd/C (excess) under hydrogen atmosphere overnight. Filtration of the catalyst and evaporation of the solvent affords the expected deprotected acid.
- 10 Lead acetate (146mg; 0.34mmol) and pyridine (0.03ml, 0.33mmol) are added to a solution of the crude solid (90mg; 0.33mmol) in dry tetrahydrofuran (THF) (25ml) under argon atmosphere. The mixture is stirred for 4 h under argon and the solid is removed by filtration. The crude
- 15 material is washed with ethyl acetate (EtOAc) and purified by chromatography on silica gel. This affords the pure dioxolane derivative.

## STEP 2

- 20 Preparation of 1-[2-benzoyloxy methyl-1,3-dioxolan-4-yl] cytosine.



- 25 A mixture of N<sup>4</sup>-acetylcytosine (124mg; 0.75mmol), dry hexamethyl disilazane (20ml) and ammonium sulfate (2-3mg; catalyst) is refluxed for 5 h. under an argon atmosphere. The clear solution is cooled to room temperature and the solvent evaporated under reduced pressure. The resulting

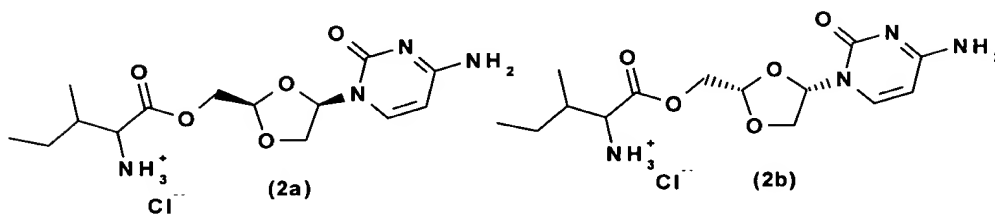
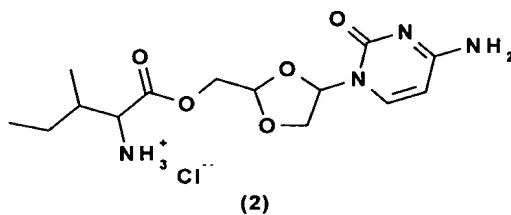
5 residue is dissolved in dry dichloromethane (15ml). A  
solution of the dioxolane derivative obtained in step 1  
(102mg; 0.55mmol) in dry dichloromethane (10ml) and  
iodotrimethyl silane (0.076ml; 0,54mmol) is added to the  
silylated cytosine. The resulting mixture is stirred for  
10 4 h. and worked-up by treating the solution with a 5%  
solution of sodium bicarbonate. The solvent of the  
resulting organic layer is evaporated under reduced  
pressure. The crude material is purified by  
chromatography on silica gel to give the expected  
15 nucleoside derivative.

### STEP 3

1-[2-hydroxymethyl-1,3-dioxolan-4-yl] N-  
20 [(dimethylamino)methylene] cytosine (268 mg; 1mmol) is  
dissolved in dichloromethane (10 ml). To this solution is  
added dicyclohexylcarbodiimide (206 mg; 1 mmol); 4-  
(dimethylamino)-pyridine (12 mg; 0.1 mmol); and Boc-  
proline (215 mg; 1mmol) at 0°C. The reaction is stirred at  
25 this temperature overnight. Insoluble is filtered off and  
the solvent is evaporated to dryness. The solid is  
redissolved in dry ether (15 ml) and the solution is  
bubbled with HCl gas at 0°C for ten minutes. The reaction  
is kept at room temperature for 2 h.. The white  
30 precipitate is filtered and dried.

5 **EXAMPLE 2**

Preparation of 2-(isoleucinyloxymethyl)-4-cytosin-1''-yl-1,3-dioxolan hydrochloride salt (2, 2a, and 2b)



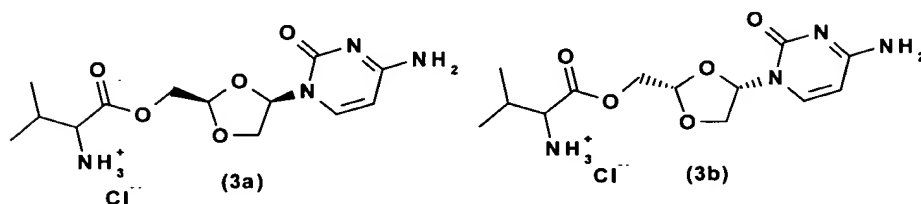
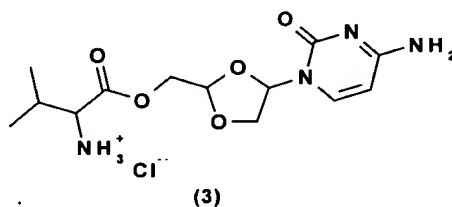
10

The above compound is synthesized according to the procedure described in example 1 except that proline is replaced by isoleucine.

15

5 **EXAMPLE 3**

Preparation of 2-(leucinyloxymethyl)-4-cytosin-1''-yl-1,3-dioxolane hydrochloride salt (3, 3a, and 3b)

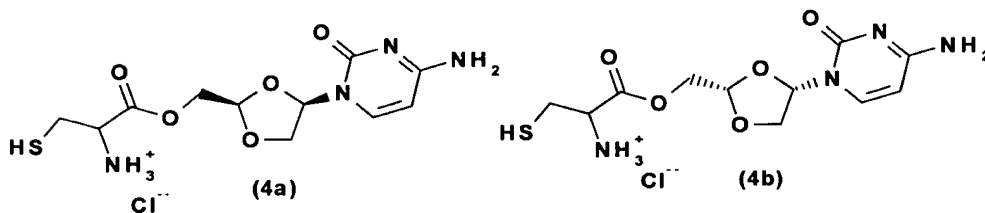
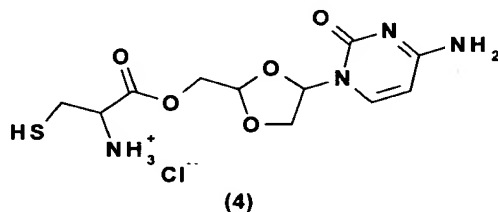


10

The above compound is synthesized according to the procedure described in example 1 except that proline is replaced by leucine.

15 **EXAMPLE 4**

Preparation of 2-(cysteinylloxymethyl)-4-cytosin-1''-yl-1,3-dioxolane hydrochloride salt (4, 4a, and 4b)



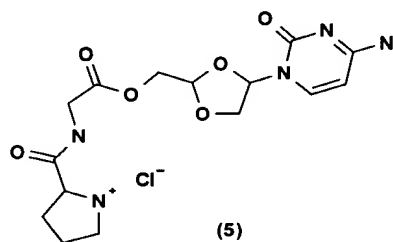
20

- 5 The above compound is synthesized according to the procedure described in example 1 except that proline is replaced by cysteine.

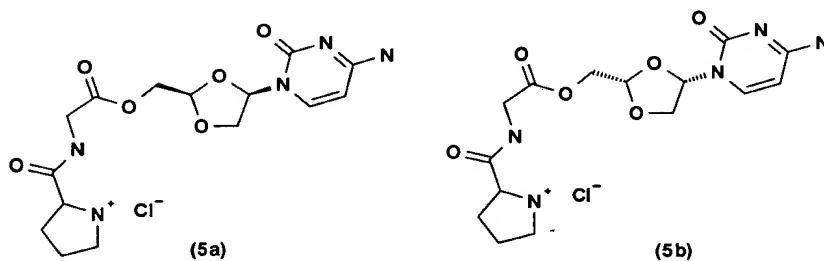
**EXAMPLE 5**

- 10 **Preparation of 2-(prolylglycinyloxymethyl)-4-cytosin-1''-yl-1,3-dioxolane hydrochloride salt (5, 5a, and 5b)**

15



25

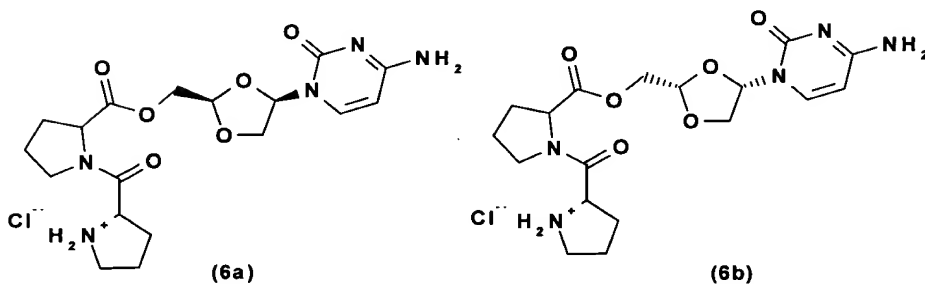
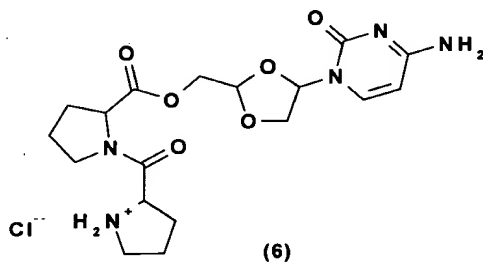


35

The compound is synthesized according to the procedure described in example 1 except that proline is replaced by prolylglycine.

5 EXAMPLE 6

Preparation of 2-(prolylprolynymethoxy)-4-cytosin-1''-yl-1,3-dioxolane hydrochloride salt (6, 6a, and 6b)



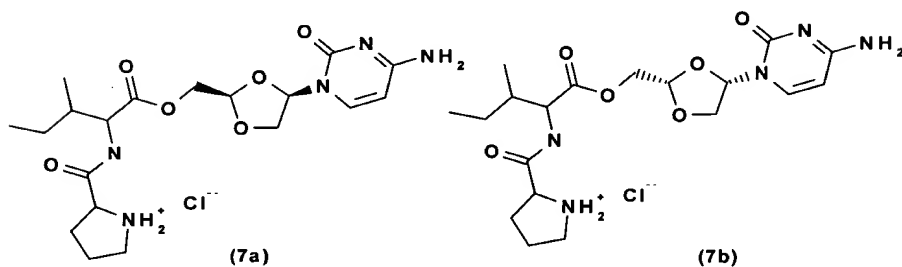
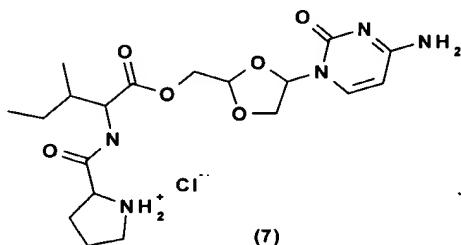
10

The above compound is synthesized according to the procedure described in example 1 except that proline is replaced by prolylproline.

15

5 **EXAMPLE 7**

Preparation of 2-(prolylleucinyloxymethyl)-4-cytosin-1''-yl-1,3-dioxolane hydrochloride salt (7 7a, and 7b)



10

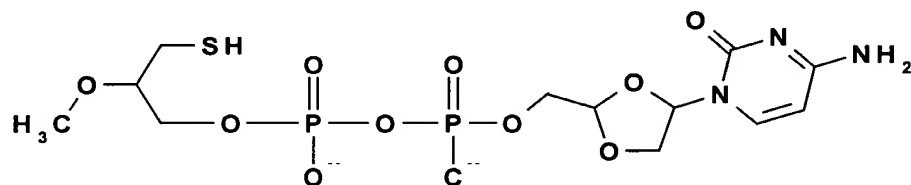
The above compound is synthesized according to the procedure described in example 1 except that proline is replaced by prolylleucine.

15

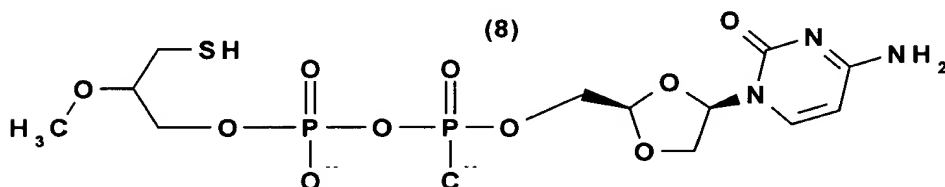
5 EXAMPLE 8

Preparation of 2-(1'-methylthio-2'-O-methyl-3'-glycerolphosphonate)-4-cytosin-1''-yl-1,3-dioxolane (8  
8a, and 8b)

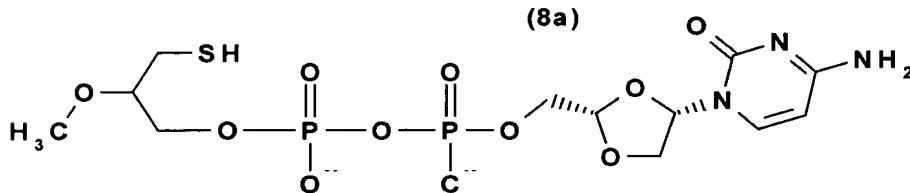
10



(8)



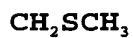
(8a)



(8b)

Step 1

15 Preparation of 1-methylthio-2-O-methyl-3  
glycerolphosphonate



|

20  $\text{CHOCH}_3$ 

|



5  $\text{CH}_2\text{OP}(\text{O})(\text{OH})_2$ 

To an ice-cold mixture of Phosphorus oxychloride (445 mg; 2.9 mmol) and hexanes (5 ml) is added dropwise triethyl  
 10 amine (295.35 mg; 2.9 mmol) in hexanes (5 ml). To this mixture is added dropwise a solution of dried 1-methylthio-2-O-methyl 3-glycerol (98 mg; 1.9 mmol) in toluene (100 ml) at 0-5°C over a period of 1.5 h, and then the mixture is stirred at room temperature overnight.  
 15 Water is added to the mixture and the organic layer is evaporated to give the desired product.

Step 2

20 Preparation of 2-(1'-methylthio-2'-O-methyl-3'-glycerolphosphonate)-4-cytosin-1''-yl-1,3-dioxolane (8a, and 8b)

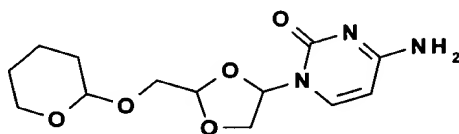
The phosphonate prepared in the first step (242 mg; 0.39  
 25 mmol) is dissolved in pyridine (10 ml). To this solution is added the dioxolane monophosphate morpholidate (198 mg; 0.31 mmol) and the mixture is stirred at room temperature for three days. Solvent is evaporated and the residue was purified by ion exchange column.

5

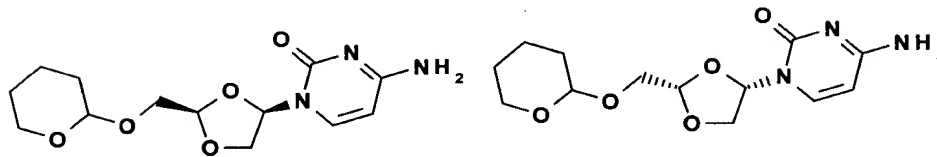
**EXAMPLE 9**

Preparation of 4-cytosin-1''-yl-1,3-dioxolane-2-(tetrahydropyranylmethyl) ether (9 9a, and 9b)

10



(9)



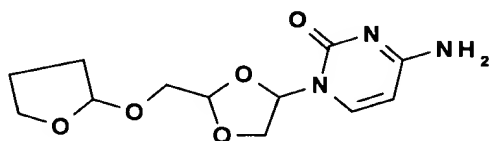
(9a)

(9b)

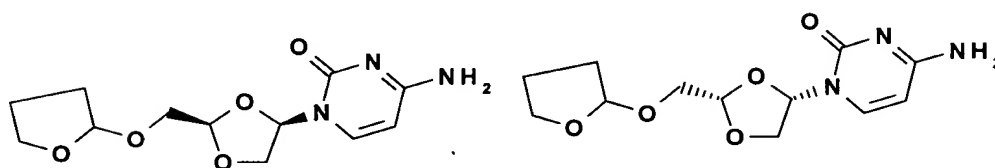
A mixture of cytosine nucleoside (684 mg; 1.9 mmol), 3,4-dihydro-2H-pyran (336 mg; 4 mmol), and p-toluene sulfonic acid (38 mg; 0.19 mmol) in dichloromethane (20 ml) is stirred for 3 h. Solvent is removed under reduced pressure and the residue is purified by chromatography.

5 **EXAMPLE 10**

Preparation of 4-cytosin-1''-yl-1,3-dioxolane-2-(tetrahydrofuranylmethyl) ether (10 10a, and 10b)



(10)



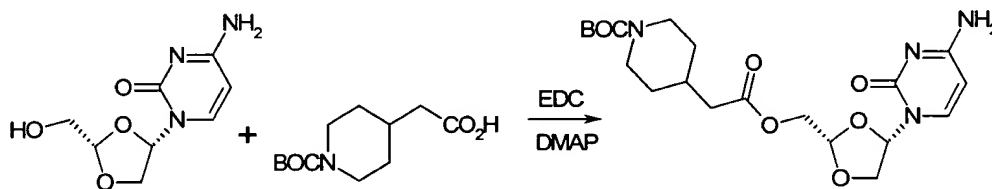
(10a)

(10b)

10

The above compound is synthesized according to the procedure described in example 9 except that 3,4-dihydro-2H-pyran is replaced by Ph<sub>2</sub>CHCO<sub>2</sub>-2-tetrahydrofuranyl.

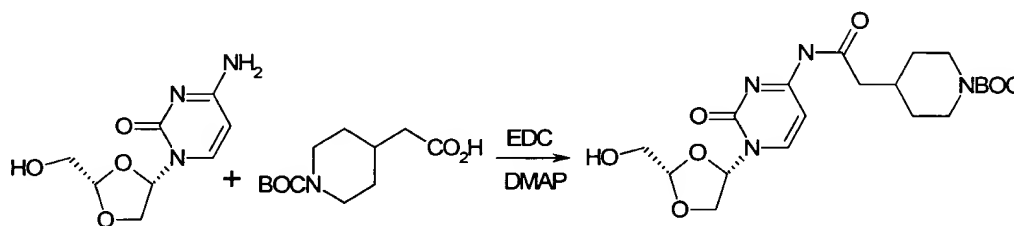
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**EXAMPLE 11**

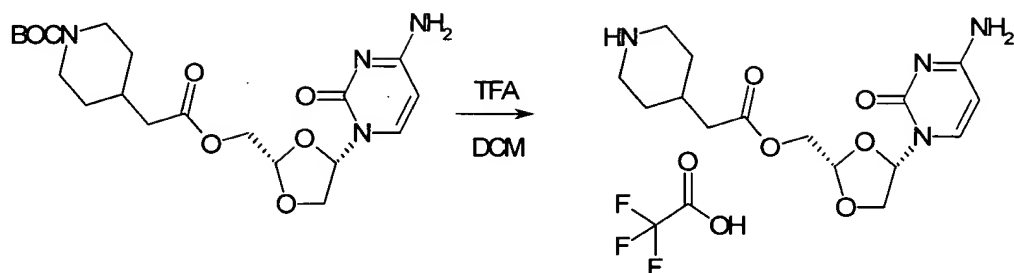
20 **Procedure:** EDC (407 mg, 2.12 mmol, 1.0eq) and DMAP (27 mg, 0.21mmol, 0.1eq) were added to a suspension of the

5 nucleoside (451 mg, 2.12 mmol, 1.0eq) and the acid (486  
 mg, 2.12mmol, 1.0eq) in DMF (10 mL) and the clear mixture  
 stirred over night at room temperature. All solvent was  
 evaporated to dryness and residue purified by  
 chromatography (from 100% ethyl acetate to 15% methanol in  
 10 ethyl acetate) 385 mg of ester was recovered.

### EXAMPLE 12



15 **Procedure:** EDC (407 mg, 2.12 mmol, 1.0eq) and DMAP (27 mg,  
 0.21mmol, 0.1eq) were added to a suspension of the  
 nucleoside (451 mg, 2.12 mmol, 1.0eq) and the acid (486  
 mg, 2.12mmol, 1.0eq) in DMF (10 mL) and the clear mixture  
 stirred over night at room temperature. All solvent was  
 20 evaporated to dryness and residue purified by  
 chromatography (from 100% ethyl acetate to 15% methanol in  
 ethyl acetate) 85 mg of amide was recovered.

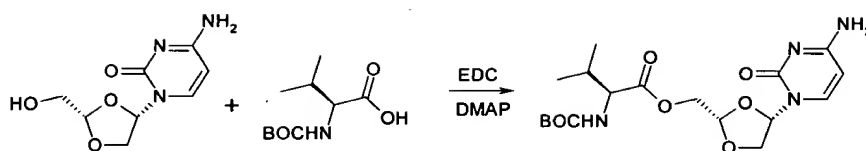
5 **EXAMPLE 13**

**Procedure:** TFA (3 mL) was added to a dichloromethane solution (7 mL) of BOC protected compound (124 mg, 0.28 mmol) and stirred for 2 hours. All solvent was evaporated to dryness. The crude was redissolved in minimal amount of methanol (0.5 mL) and slowly added to ether (10 mL) with strong agitation. The supernatant was removed and the solid dried under vacuum. 125 mg was isolated.

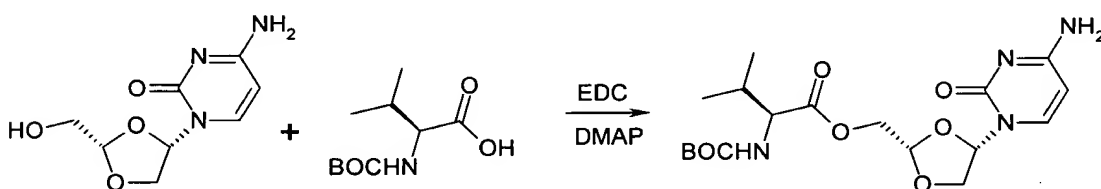
<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): 8.50 (br s, 1H), 8.25 (br s, 2H), 7.80 (d, J=7.5Hz, 1H), 6.23 (d, J=4.0Hz, 1H), 6.01 (d, J=8.0Hz, 1H), 5.19 (t, J=3.0Hz, 1H), 4.35-4.25 (m, 3H), 4.16 (m, 1H), 3.25 (d, J=13.5Hz, 2H), 2.88 (q, J=11.0Hz, 2H), 2.36 (d, J=7.0Hz, 2H), 1.95 (m, 1H), 1.81 (d, J=13.0Hz, 2H), 1.33 (q, J=10.0Hz, 2H).

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): 10.92 (s, 1H), 8.50 (br s, 1H), 8.38 (d, J=7.5Hz, 1H), 8.15 (br s, 1H), 7.22 (d, J=7.5Hz, 1H), 6.15 (m, 1H), 5.00 (s, 1H), 4.17 (d, J=4.5Hz, 2H), 3.71 (s, 2H), 3.24 (d, J=12.0Hz, 2H), 2.89 (q, J=8.5Hz, 2H), 2.39 (d, J=7.0Hz, 2H), 2.00 (br s, 1H), 1.79 (d, J=14.0Hz, 2H), 1.34 (q, 12.0Hz, 2H).

25

5 **EXAMPLE 15**

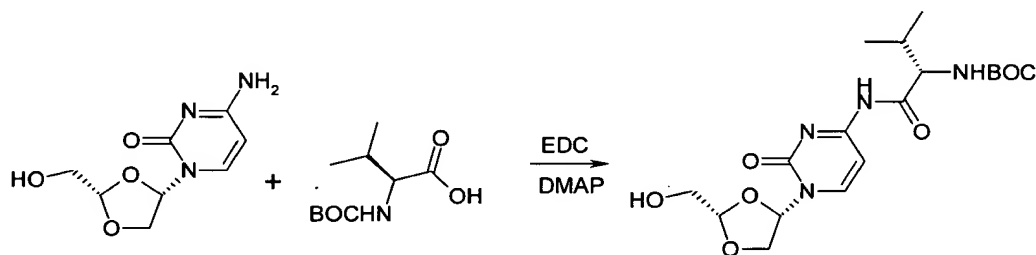
**Procedure:** EDC (512 mg, 2.67 mmol, 1.0eq) and DMAP (34 mg, 0.27 mmol, 0.1eq) were added to a suspension of the nucleoside (568 mg, 2.67 mmol, 1.0eq) and the acid (565 mg, 2.67 mmol, 1.0eq) in DMF (10 mL) and the clear mixture stirred over night at room temperature. All solvent was evaporated to dryness and residue purified by chromatography (from 100% ethyl acetate to 15% methanol in ethyl acetate) 355 mg of ester was recovered.

**EXAMPLE 16**

**Procedure:** EDC (512 mg, 2.67 mmol, 1.0eq) and DMAP (34 mg, 0.27 mmol, 0.1eq) were added to a suspension of the nucleoside (568 mg, 2.67 mmol, 1.0eq) and the acid (565

5 mg, 2.67 mmol, 1.0eq) in DMF (10 mL) and the clear mixture stirred over night at room temperature. All solvent was evaporated to dryness and residue purified by chromatography (from 100% ethyl acetate to 15% methanol in ethyl acetate) 355 mg of ester was recovered.

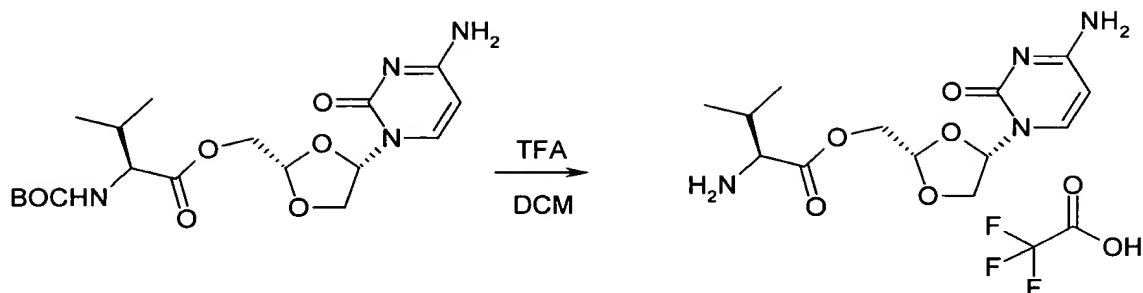
10

**EXAMPLE 17**

**Procedure:** EDC (512 mg, 2.67 mmol, 1.0eq) and DMAP (34 mg, 0.27 mmol, 0.1eq) were added to a suspension of the nucleoside (568 mg, 2.67 mmol, 1.0eq) and the acid (565 mg, 2.67 mmol, 1.0eq) in DMF (10 mL) and the clear mixture stirred over night at room temperature. All solvent was evaporated to dryness and residue purified by chromatography (from 100% ethyl acetate to 15% methanol in ethyl acetate) 102 mg of amide was recovered.

25



5 **EXAMPLE 18**

**Procedure:** TFA (3 mL) was added to a dichloromethane solution (7 mL) of BOC protected compound (127 mg, 0.31 mmol) and stirred for 2 hours. All solvent was evaporated to dryness. The crude was redissolved in minimal amount of methanol (0.5 mL) and slowly added to ether (10 mL) with strong agitation. The supernatant was removed and the solid dried under vacuum. 111 mg was isolated.

15

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ): 8.40 (br s, 2H), 8.15 (br s, 1H), 7.75 (d,  $J=7.5\text{Hz}$ , 1H), 6.27 (d,  $J=4.0\text{Hz}$ , 1H), 6.00 (d,  $J=7.5\text{Hz}$ , 1H), 5.23 (t,  $J=3.5\text{Hz}$ , 1H), 4.49 (qd,  $J=12.0\text{Hz}$ ,  $J=3.0\text{Hz}$ , 2H), 4.29 (d,  $J=10.0\text{Hz}$ , 1H), 4.19 (m, 1H), 4.04 (s, 1H), 2.14 (m, 1H), 0.95 (D,  $J=7.0\text{Hz}$ , 6H).

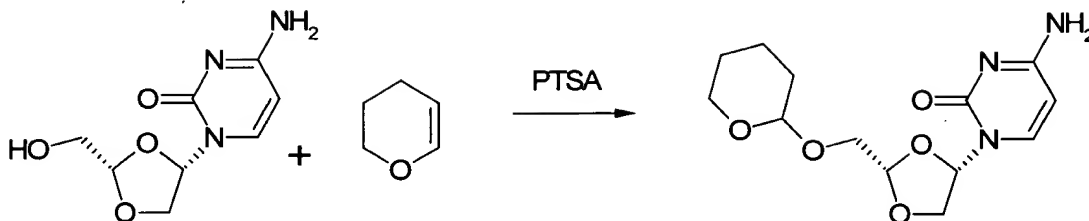
25

## 10



20

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): 8.48 (d, J=7.5Hz, 1H), 8.25 (br s, 3H), 7.17 (d, J=7.5Hz, 1H), 6.16 (d, J=4.0Hz, 1H), 5.29 (m, 1H), 5.03 (t, J=2.5Hz, 1H), 4.25-4.15 (m, 2H), 3.90 (s, 1H), 3.72 (s, 2H), 2.18 (m, 1H), 0.95 (m, 6H).

**EXAMPLE 20**

**Procedure:** Paratoluene sulfonic acid (82mg, 0.43 mmol, 1.0eq.) was added to a solution of BCH-4556 (92mg, 0.43mmol, 1.0eq.) in DMF (1mL) and 3,4-dihydropyran (3mL). The reaction was stirred for 16 hours and potassium carbonate (119mg, 0.86mmol, 2.0eq.) added and stirred for 1 hour. The solid was filtered off and the solvent evaporated to dryness. The crude was purified by flash using a gradient of 5 to 10% methanol in dichloromethane. 100mg of desired compound was isolated.

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ): 7.79 (t,  $J=8.0\text{Hz}$ , 1H), 7.18 (br d,  $J=20.0\text{Hz}$ , 2H), 6.20 (m, 1H), 5.71 (d,  $J=7.0\text{Hz}$ , 1H), 5.09 (m, 1H), 4.68 (m, 1H), 4.09 (m, 2H), 3.86 (m, 1H), 3.80-3.65 (m, 2H), 3.48 (m, 1H), 1.80-1.60 (m, 2H), 1.60-1.45 (m, 4H).

5

**EXAMPLE 21**

**Preparation of Cis-L-2-[2''-cyanoethyl methoxy- L-phenylalaninylphosphoramidyloxymethyl-4-(cytosin-1'-yl)]-1,3-dioxolane**

10

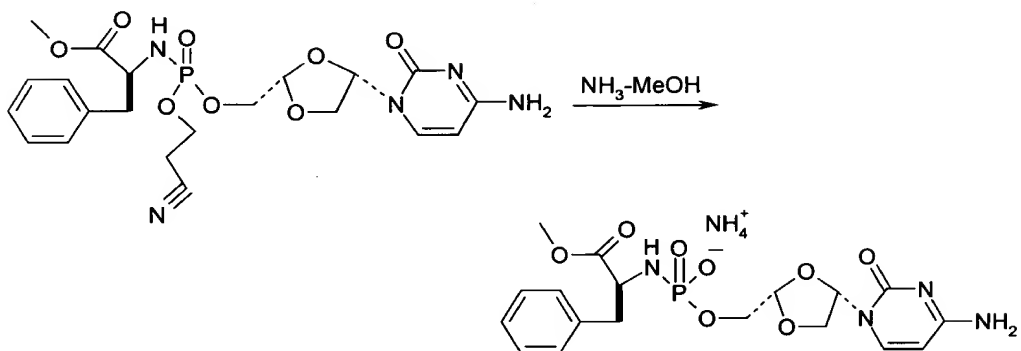
**Procedure:** Dry BCH 4556( dimethylaminomethylene derivative, 0.1 g, 0.373 mmol) was dissolved in dry DMA (2 ml) under nitrogen and cooled in an ice bath.

Diisopropylethylamine(0.2 ml) and 2,cyanoethyl-N,N-  
15 diisopropylchlorophosphoramidite (0.17 ml, 1.12 mmol) were added in respective order. After 1 hour <sup>1</sup>Tetrazole (0.1 g, 1.49 mmol) was added and after 10 minutes dry methanol (0.05 ml) was introduced. The reaction mixture was allowed to warm to room temperature over 2 hours. L-  
20 phenylalanine methyl ester (hydrochloride, 0.39 g, 2.18 mmol) and iodine (0.19 g, 0.746 mmol) were added in respective order. Combined mixture was allowed to stir for 2 hours and excess iodine was quenched with saturated sodium thiosulphate solution. It was evaporated to  
25 dryness and the residue was extracted with dichloromethane, washed with brine and dried over anhydrous MgSO<sub>4</sub>. After evaporation the crude product was purified on a flash silica gel column which was eluted with a mixture of dichloromethane and methanol (ratio  
30 10:1). Tare of the title compound was 0.072 g.

- 5  $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$ : 7.95 (1H, d); 6.7 (1H, dd); 6.2 (1H, dd); 5.01 (1H, s); 4.9-2.5 (m, 14H) ppm.

Appearance oil

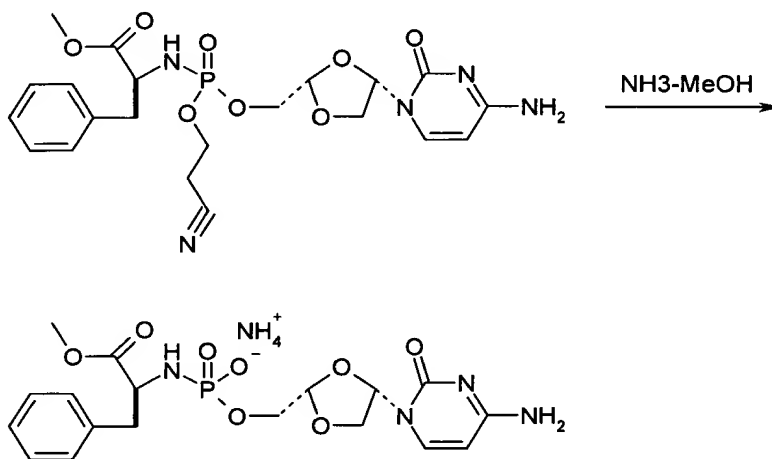
- 10 **Ref.** Abraham, T.W.; Wagner, C.R. Nucleosides &



Nucleotides, 13(9), 1891-1903 (1994)

5 **EXAMPLE 22****Preparation of Cis-L-2-methoxy-L-phenylalaninylphosphoro-  
amidyloxymethyl-4-(cytosin-1'-yl)]-1,3-dioxolane****Ammonium salt**

10 **Ref** Abraham, T.W.; Wagner, C.R. Nucleosides & Nucleotides, 13(9), 1891-1903 (1994)



Appearance Foam

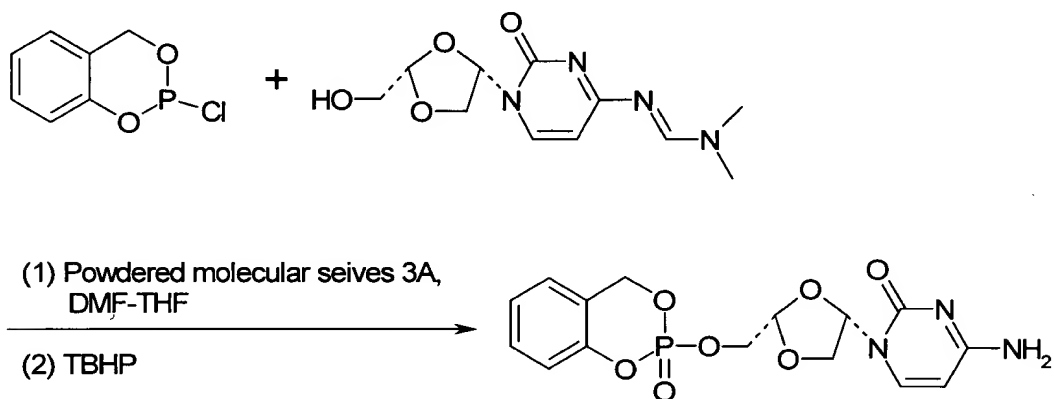
Procedure: Dry Cis-L-2-[2''-cyanoethyl methoxy- L-phenylalaninylphosphoroamidyloxymethyl-4-(cytosin-1'-yl)]-1,3-dioxolane (0.072g, 0.128 mmol) was dissolved in dry methanol (9.7 ml) and mixed with a saturated solution of ammonia in dry methanol (5.8 ml). Combined mixture was allowed to stir for 1 hour. Solvent was evaporated and the crude product was purified on a silica gel column which was eluted with a mixture of dichloromethane and methanol (ratio 2:1). Tare of the title compound was 0.031g.

131  
5  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$ : 8.15 (1H, d); 7.2 (5H, m);  
6.25 (1H, t); 6.05 (1H, d); 5.08 (1H, s); 4.05 (5H, m);  
3.55 (3H, s); 3.0 (2H, qq) ppm.

UV:  $\lambda_{\text{max}}$  (MeOH) 272 nm.

10

MS: m/e 453.2

5 **EXAMPLE 23****Preparation of Cis-1-Cyclosaligenyl-2-oxymethyl-[(4-cytosin-1'-yl)-1,3-dioxolane]-phosphate diastereomers**

10

**Procedure:** Dry BCH 4556( dimethylaminomethylene derivative, 0.05g, 0.1865 mmol) was dissolved in dry DMF (2 ml) and dry THF (1 ml). It was cooled to -40° C in an argon atmosphere. Freshly activated powdered

15 molecular sieves (0.05g) were added. Cyclic saligenylchloroposphanes (0.071g, 0.373 mmol) was dissolved in dry THF (0.5 ml) and introduced over 30 minutes. Combined mixture was stirred at -40° C for another half an hour. Tert-Butylhydroperoxide (3 M

20 solution in 2,2,4-trimethylpentane, 0.125 ml) was added. After stirring for half an hour, the reaction mixture was allowed to warm to room temperature. The solvent was evaporated and the crude product was extracted with ethyl acetate. It was purified on a silica gel column using a



5 mixture of ethyl acetate and methanol (ratio 5:2).

Further purification and the separation of diastereomers was carried on reverse phase HPLC.

$^1\text{H}$  NMR (400MHZ, DMSO-D<sub>6</sub>)  $\delta$  : 8.25 (1H, d); 7.4 (5H, m);  
10 6.15 (1H, t); 5.75 (1H, d), 5.5 (2H, m); 5.2 (1H, s); 4.2 (4H, m)  
ppm.

UV :  $\lambda_{\text{max}}$  (MeCN) 277nm

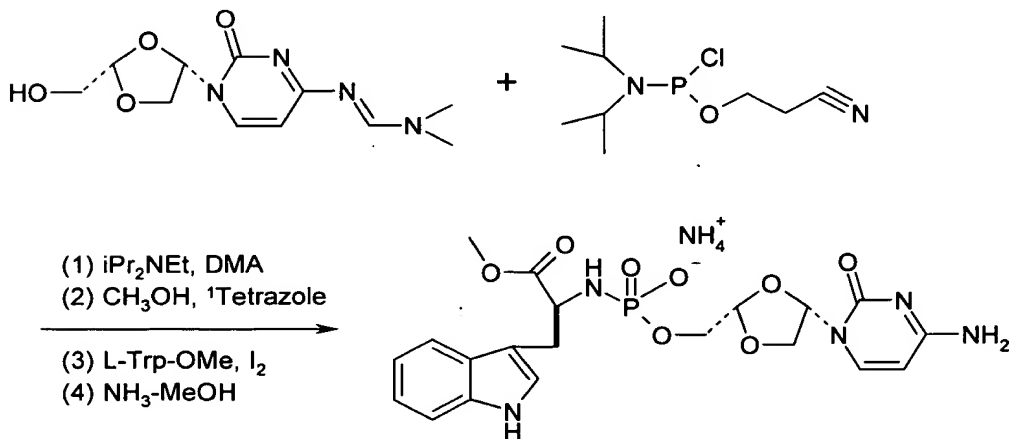
15 MS : m/e 381

Ref Meier, C.; Knispel, T.;  
Marquez, V.E.; Siddiqui, M.A.; De  
Clercq, E.; Balzarini, J.  
J. Med. Chem. **1999**, 42, 1615-1624.

Appearance Foam

#### EXAMPLE 24

20 Preparation of Cis-L-2-methoxy-L-tryptophanylphosphoramidylloxymethyl-4-(cytosin-1'-yl)]-1,3-dioxolane Ammonium salt



5.

**Procedure:** Dry BCH 4556 (dimethylaminomethylene derivative, 0.16 g, 0.597 mmol) was dissolved in dry DMA (3.2 ml) under nitrogen and cooled in an ice bath. Diisopropylethylamine (0.32 ml) and 2, cyanoethyl-N,N-  
10 diisopropylchlorophosphoramidite (0.27 ml, 1.79 mmol) were added in respective order. After 1 hour <sup>1</sup>Tetrazole (0.16 g, 2.38 mmol) was added and after 10 minutes dry methanol (0.08 ml) was introduced. The reaction mixture was allowed to warm to room temperature over 2 hours. L-  
15 tryptophan methyl ester (hydrochloride, 0.74 g, 3.5 mmol) and iodine (0.32 g, 1.2 mmol) were added in respective order. Combined mixture was allowed to stir for 2 hours and excess iodine was quenched with saturated sodium thiosulphate solution. It was evaporated to dryness and  
20 the residue was extracted with dichloromethane, washed with brine and dried over anhydrous MgSO<sub>4</sub>. After evaporation the crude product was purified on a flash silica gel column which was eluted with a mixture of dichloromethane and methanol (ratio 5:1).

25

The product was dissolved in dry methanol (15 ml) and mixed with a saturated solution of ammonia in dry methanol (9.3 ml). Combined mixture was allowed to stir for 1 hour. Solvent was evaporated and the crude product was  
30 purified on a silica gel column which was eluted with a

5 mixture of dichloromethane and methanol (ratio 2:1). Tare of the title compound was 0.016 g.

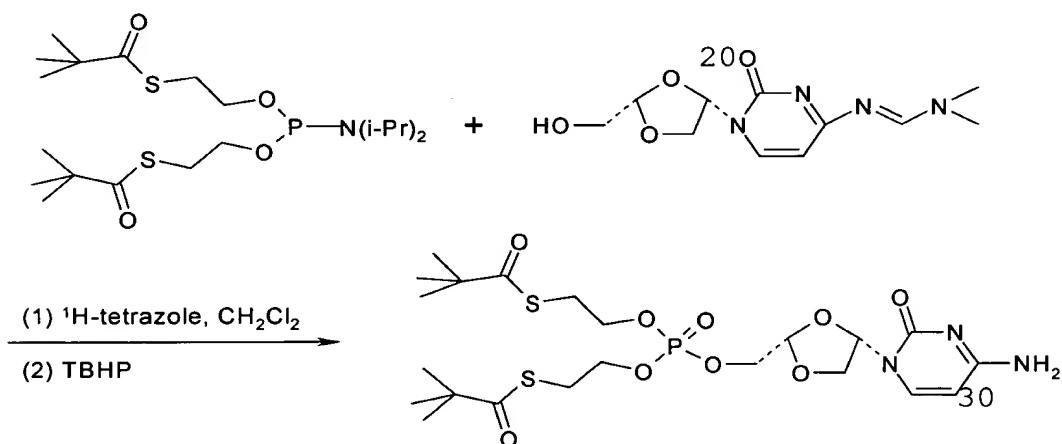
$^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$ : 8.1 (1H, d); 7.2 (5H, m); 6.2 (1H, t); 5.95 (1H, d); 5.05 (1H, s); 4.1 (5H, m); 3.35 (5H, m) ppm.

10

### EXAMPLE 25

15

#### Preparation of (2S,4S)-2-[bis(S-pivaloyl-2-thioethyl)phosphono]-4-cytosin-1'-yl-1,3-dioxolane



**Procedure:** Dry BCH 4556 ( dimethylaminomethylene derivative, 0.095 g, 0.354 mmol) was mixed with bis-(S-pivaloyl-2-thioethyl)-N,N-diisopropylphosphoramidite (0.18 g, 0.5 mmol, prepared following the procedure described in P.R.No.27-25) and dissolved in dry dichloromethane (15 ml).  $^1\text{H}$ -tetrazole (0.075 g, 1.06 mmol) was added and the

5 combined solution was stirred under nitrogen atmosphere at  
room temperature for 1 hour. It was cooled to  $-40^{\circ}\text{C}$  and  
treated with tert-butylhydroperoxide (3 M solution in  
2,2,4-trimethylpentane, 0.25 ml). Reaction mixture was  
allowed to warm up to room temperature during overnight.  
10 Solvent was evaporated and the residue was purified on a  
silica gel column using a mixture of ethyl acetate and  
methanol (ratio 40:1). Tare of the title product 0.055 g.

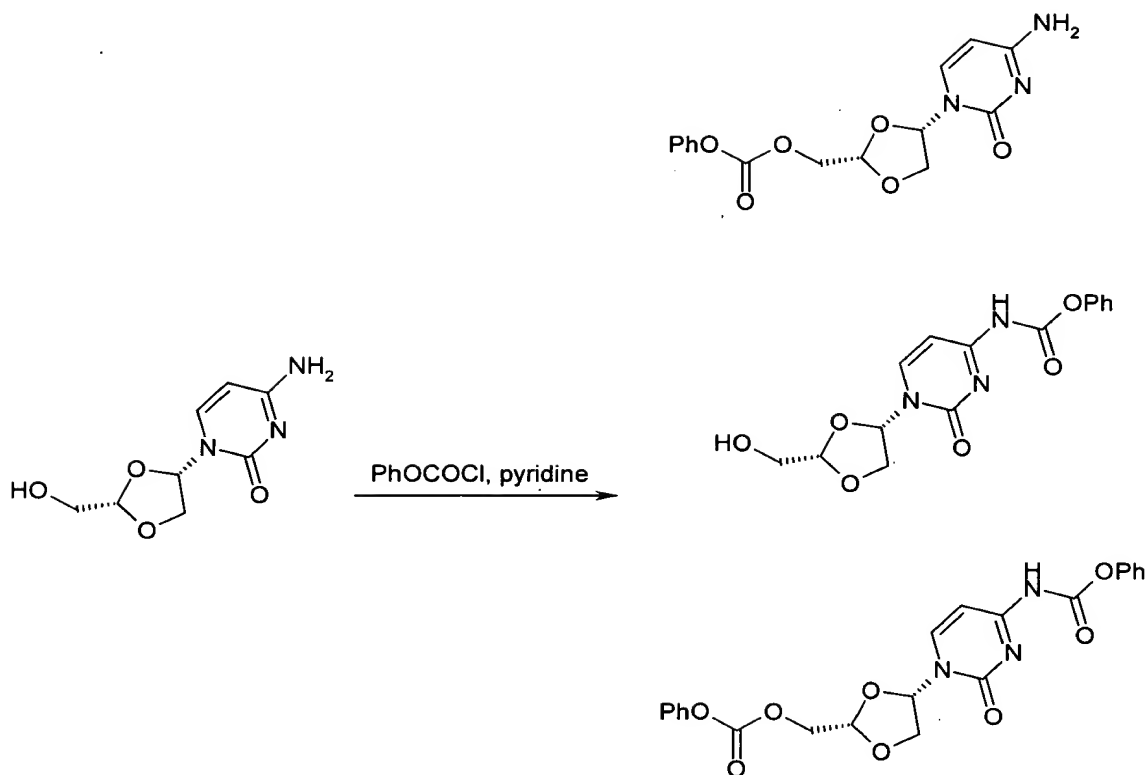
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 7.8 (1H, d); 6.3 (1H, t); 5.95 (1H,  
15 d); 4.18 (8H, m); 3.15 (4H, m); 1.2 (18H, s) ppm.

$^{31}\text{P}$  NMR (16 MHz,  $\text{CDCl}_3$ )  $\delta$ : -0.13

UV :  $\lambda_{\text{max}}$  (MeCN) 271nm

20

MS : m/e 582.4

5 Example 2610 Typical procedure for the reaction with alkyl(or aryl) chloroformate

BCH-4556 (1 mmole) and phenyl chloroformate (1 mmole) were stirred for 24 hours in 10 mL of pyridine. Pyridine was then evaporated, the residue was dissolved in 10 mL of water and extracted with dichloromethane. The organic phase is dried on sodium sulfate evaporated and the residue is chromatographed on silica gel eluting first with 50/50 ethyl acetate/hexane, then ethyl acetate and

5 finally with 10% MeOH/dichloromethane. The three  
compounds were isolated separately. The final products  
can be further purified using reverse phase preparative  
HPLC.

10

15

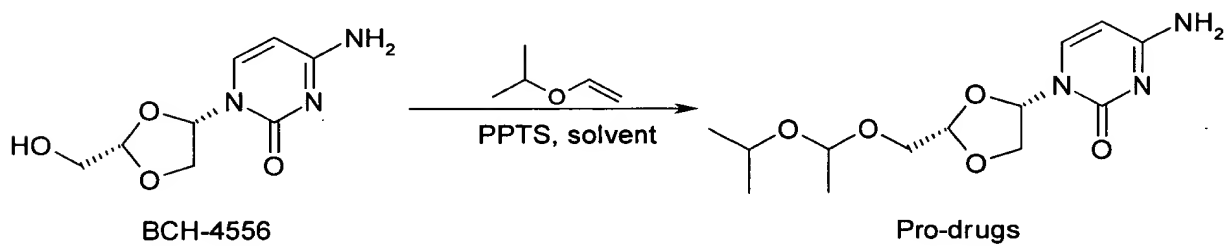
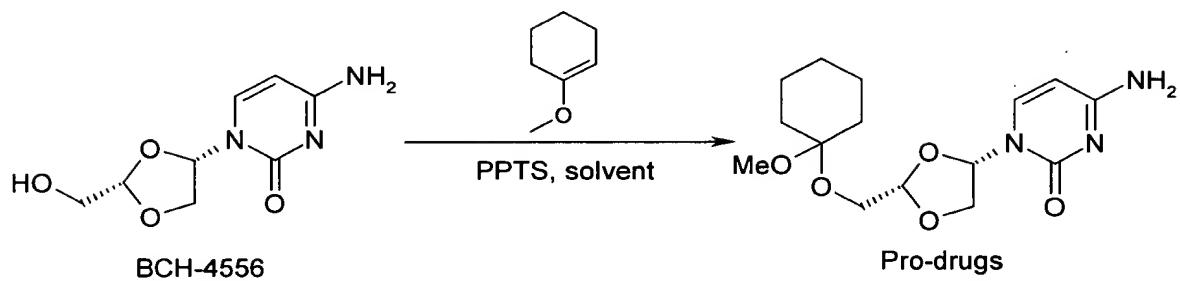
20

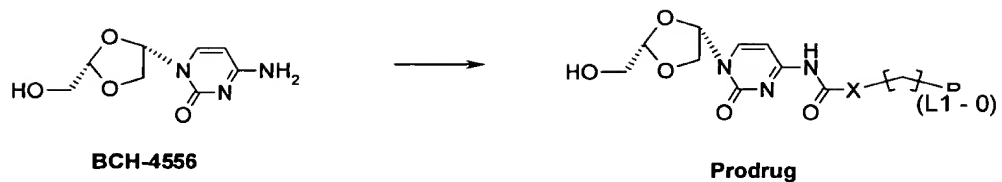
25

30

Example 27

The following are additional synthesis reaction schemes.



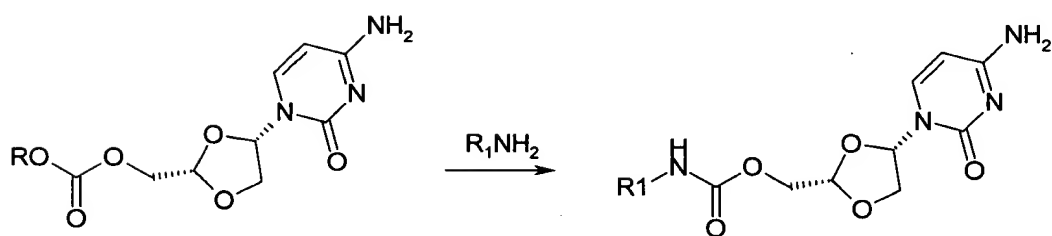
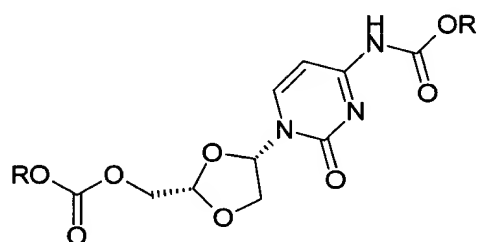
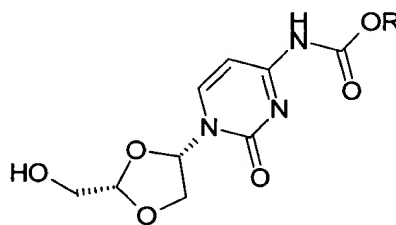
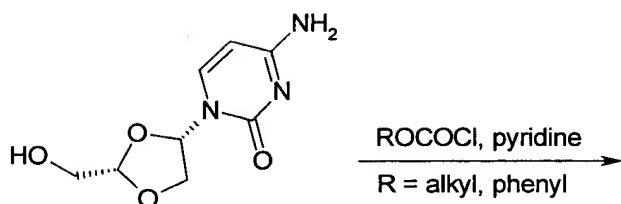
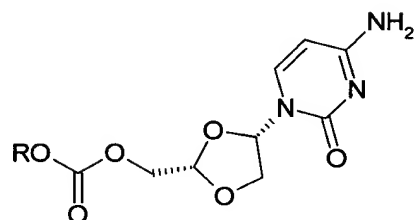


$n = 3, 4, 5$ ;  $X = \text{CH}_2$ ;  $R = \text{CH}_3$

$n = 3, 4, 5$ ;  $X = \text{O}$ ;  $R = \text{CH}_3$

$n = 3, 4, 5$ ;  $X = \text{CH}_2$ ;  $R = \text{N}(\text{CH}_3)_2$

$n = 3, 4, 5$ ;  $X = \text{O}$ ;  $R = \text{N}(\text{CH}_3)_2$



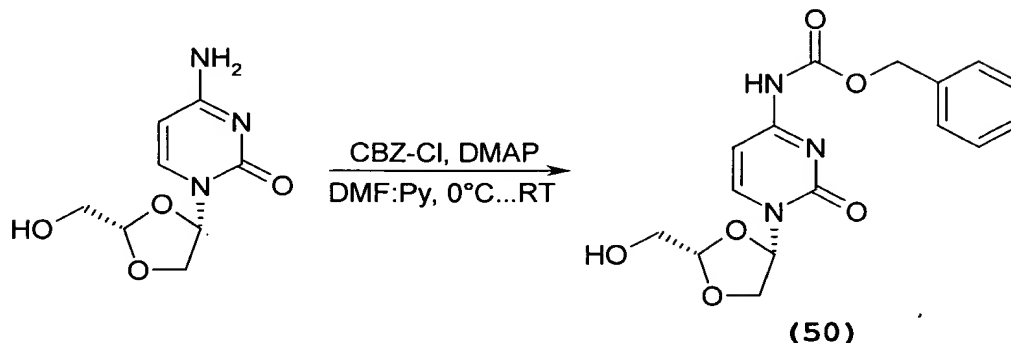
$R = \text{phenyl}$



5

EXAMPLE 28**Preparation of [1-(2-Hydroxymethyl-[1,3]dioxolan-4-yl)cysosyl]carbamic acid benzyl ester**

10



## Procedure:

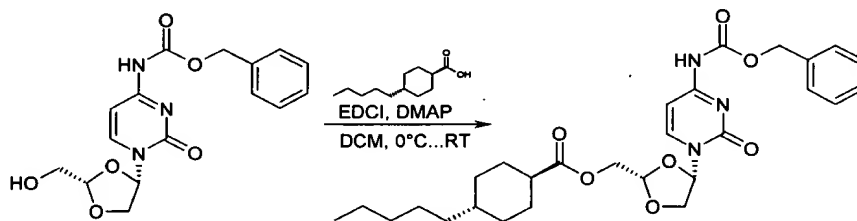
Benzyloxychloroformate (0.80 mL, 5.6 mmol) was added dropwise to a 0°C solution of BCH-4556 (955 mg, 4.48 mmol) and DMAP (657 mg, 5.38 mmol) in dimethylformamide and pyridine and stirred at room temperature for 18h. The reaction mixture was concentrated in vacuo. The oil obtained was partitioned between water (20mL) and dichloromethane (30mL). Aqueous layer was extracted with DCM. Organic layers were combined, dried over MgSO<sub>4</sub>, filtered and concentrated to a yellow gum. The crude residue was purified by silica gel biotage (40S) (100 % DCM to 10 % MeOH: 90 % DCM) to give 837 mg (54 % yield) of [1-(2-Hydroxymethyl-[1,3]dioxolan-4-yl)cysosyl]carbamic acid benzyl ester as a white powder, M.F. C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>6</sub>, M.W. 347.33.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>), δ ppm: 8.44 (d, 1H, J = 7.4Hz), 7.39-7.37 (m, 5H), 7.25 (m, 1H), 6.18 (d, 1H, J = 3.9Hz), 5.21 (s, 2H), 5.13-5.12 (m, 1H), 4.34 (d, 1H, J = 10.1Hz), 4.25 (dd, 1H, J = 5.2, 10.1Hz), 4.01-3.97 (m, 2H). MS: ES<sup>+</sup> 348.4 (M+1), ES<sup>-</sup> 346.3 (M-1).

35

5 EXAMPLE 29

**Preparation of [1{2-(trans-4-pentylcyclohexylcarboxy)oxy-methyl-[1,3]dioxolan-4-yl}cysosyl]carbamic acid benzyl ester**



10

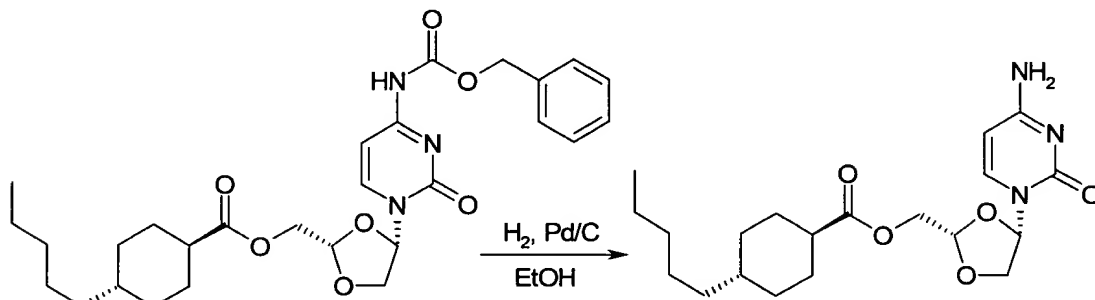
**Procedure:**

EDCI (1.66g, 8.64 mmol) was added to a 0°C solution of [1-(2-Hydroxymethyl-[1,3]dioxolan-4-yl)cysosyl]carbamic acid benzyl ester (2.5 g, 7.20 mmol), DMAP (1.05 g, 8.64 mmol) and trans-4-pentylcyclohexylcarboxylic acid (1.71g, 8.64 mmol) in dichloromethane and stirred at room temperature for 18h. The reaction was washed with HCl, saturated NaHCO<sub>3</sub> and brine. Organic layer was separated, dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo. The crude residue was purified by silica gel biotage (40M) (100 % DCM to 3 % MeOH: 97 % DCM) to give 3.92 g (100 % yield) of [1{2-(trans-4-pentylcyclohexylcarboxy)oxymethyl-1,3]dioxolan-4-yl}cysosyl]carbamic acid benzyl ester as a white powder, M.F. C<sub>28</sub>H<sub>37</sub>N<sub>3</sub>O<sub>7</sub>, M.W. 527.62.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>), δ ppm: 8.15 (d, 1H, J = 7.4Hz), 7.39-7.31 (m, 5H), 7.30 (d, 1H, J = 7.4Hz), 6.19 (d, 1H, J = 4.1Hz), 5.24-5.22 (m, 3H), 4.55 (dd, 1H, J = 3.3, 12.7Hz), 4.32-4.22 (m, 3H), 2.31-2.23 (m, 1H), 1.99-1.91 (m, 2H), 1.85-1.80 (m, 2H), 1.49-1.37 (m, 1H), 1.31-1.16 (m, 10H), 0.98-0.86 (m, 5H).

30

5

EXAMPLE 30**Preparation of trans-4-Pentylcyclohexylcarboxylic acid 4-cytosyl-[1,3]dioxolan-2-ylmethyl ester**

10

**Procedure:**

[1{2-(trans-4-pentylcyclohexylcarboxy)oxymethyl-  
 15 [1,3]dioxolan-4-yl}cysosyl]carbamic acid benzyl ester  
 (3.8g, 7.20 mmol) and Pd/C 10% (600 mg) were suspended in  
 ethanol and EtOAc. The reaction was treated three times  
 with a vacuum-nitrogen sequence and left under nitrogen.  
 It was then submitted to a vacuum-hydrogen sequence and  
 20 the reaction stirred under hydrogen for 3 hrs. The  
 reaction was filtered on a celite pad and washed with EtOH  
 and the solution concentrated in vacuo. The crude solid  
 was purified by silica gel biotage (40M) to give 2.44 g  
 (86 % yield) of trans-4-pentylcyclohexylcarboxylic acid 4-  
 25 cytosyl-[1,3]dioxolan-2-ylmethyl ester as a white powder,  
 M.F.  $C_{20}H_{31}N_3O_5$  , M.W. 393.49.

$^1H$  NMR (400 MHz,  $CD_3OD$ ),  $\delta$  ppm: 7.85 (d, 1H,  $J = 7.5Hz$ ),  
 6.23 (dd, 1H,  $J = 1.9, 5.3Hz$ ), 5.90 (d, 1H,  $J = 7.5Hz$ ),  
 30 5.21 (t, 1H,  $J = 2.7Hz$ ), 4.43 (dd, 1H,  $J = 2.7, 12.7Hz$ ),  
 4.29 (dd, 1H,  $J = 2.6, 12.7Hz$ ), 4.25-4.17 (m, 2H), 2.29-  
 2.22 (m, 1H), 1.95-1.89 (m, 2H), 1.83-1.80 (m, 2H), 1.44-  
 1.19 (m, 11H), 0.99-0.88 (m, 5H).

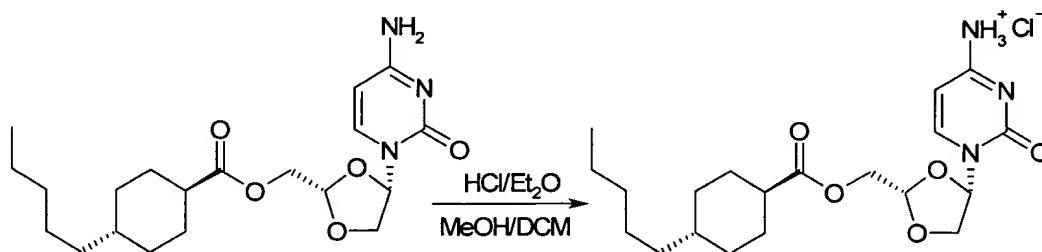
35

5 EXAMPLE 31

**Preparation of trans-4-Pentylcyclohexylcarboxylic acid 4-cytosyl-[1,3]dioxolan-2-ylmethyl ester hydrochloride salt**

10

(264)



Procedure:

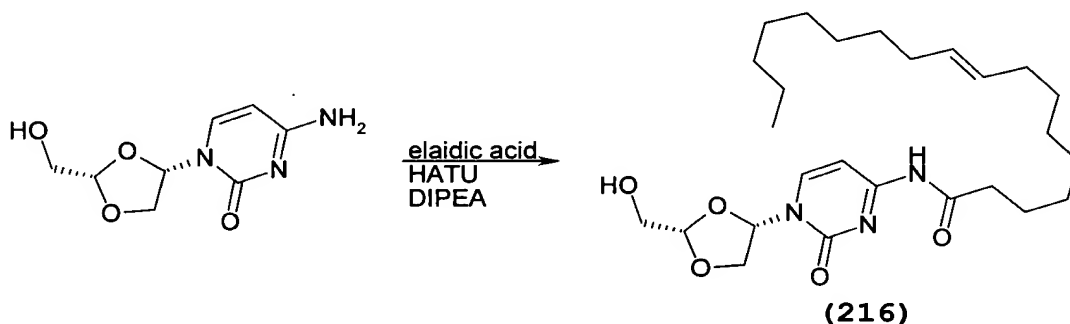
A 1M ether solution of HCl was added to a 0°C solution of trans-4-pentylcyclohexylcarboxylic acid 4-cytosyl-1,3]dioxolan-2-ylmethyl ester in a 1:1 mixture of MeOH and DCM and the reaction stirred at room temperature for 1.5h. Solvent was then removed in vacuo to give 99% yield of trans-4-pentylcyclohexylcarboxylic acid 4-cytosyl-1,3]dioxolan-2-ylmethyl ester hydrochloride salt as a white powder, M.F. C<sub>20</sub>H<sub>31</sub>N<sub>3</sub>O<sub>5</sub> HCl, M.W. 429.95.

20

<sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD), δ ppm: 8.13 (d, 1H, J = 7.8Hz), 6.26 (dd, 1H, J = 1.5, 5.5Hz), 6.11 (d, 1H, J = 7.8Hz), 5.24 (t, 1H, J = 2.8Hz), 4.47 (dd, 1H, J = 2.8, 12.6Hz), 4.40 (dd, 1H, J = 1.2, 10.3), 4.31 (dd, 1H, J = 2.8, 12.6Hz), 4.22 (dd, 1H, J = 5.5, 10.3Hz), 2.31-2.25 (s, 1H), 1.96-1.91 (m, 2H), 1.85-1.82 (m, 2H), 1.42-1.19 (m, 11H), 0.96-0.88 (m, 5H).

5 EXAMPLE 32

**Preparation of Octadecen-9-enoic[1-(2-hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-amide**



Procedure:

15 The starting material (BCH-4556, 86,3 mg, 0,405 mmole) is dissolved in DMF. Diisopropylethyl amine is then added (0,486 mmole, 1,2 eq) followed by the acid (0,521 mmole, 1,3 eq.). CH<sub>2</sub>Cl<sub>2</sub> is then added to put everything in solution. HATU (168 mg, 0,446 mmole, 1,1 eq) is then added and the solution is stirred for 2 days. A saturated aqueous solution of NaHCO<sub>3</sub> is then added and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic phase is evaporated and the residue is purified by Biotage with a Flash 12S column using 2% MeOH in CH<sub>2</sub>Cl<sub>2</sub> followed by 4% MeOH in CH<sub>2</sub>Cl<sub>2</sub>. The desired fractions are recovered and evaporated to afford 39% of the desired compound.

25

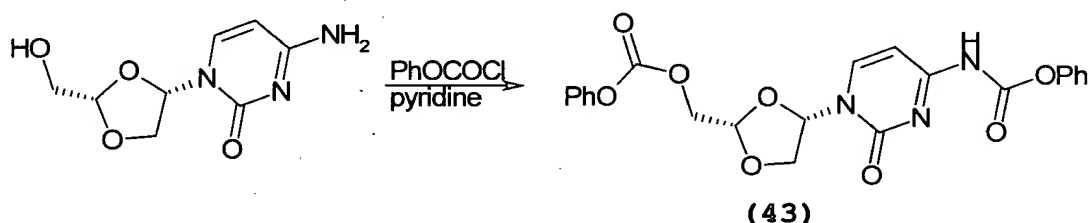
<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8,98 (s, 1H), 8,46 (d, 1H, J=7,6 Hz), 7,42 (d, 1H, J=7,6 Hz), 6,18 (dd, 1H, J=5,2 and 1,4 Hz), 5,36 (m, 2H), 5,11 (t, 1H, J=1,8 Hz), 4,31 (dd, 1H, J=10,2 and 1,3 Hz), 4,23 (m, 1H), 3,86 (s, 2H), 3,02 (s, 1H), 2,44 (t, 2H, J=7,6 Hz), 1,94 (m, 4H), 1,64 (m, 2H), 1,43 (m, 20H), 0,86 (t, 3H, J=6,9 Hz).

30

5 EXAMPLE 33

Preparation of Carbonic acid 4-(2-oxo-4-phenoxycarbonylamino-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethyl ester phenyl ester

10



## Procedure:

15 The starting material (BCH-4556, 105 mg, 0,493 mmole) is dissolved in 2 mL of pyridine and cooled to 0 °C. Phenyl chloroformate (68  $\mu$ L, 0,542 mmole, 1,1 eq.) is added and the reaction mixture is warmed to room temperature and stirred overnight. The solvent is then evaporated and

20 water is added. The aqueous phase is extracted with methylene chloride. The organic extracts are dried over  $\text{Na}_2\text{SO}_4$  and evaporated. The residue is purified by Biotage with 50/50 AcOEt/Hexane then AcOEt followed by 10% MeOH/ $\text{CH}_2\text{Cl}_2$ . The fractions containing the fastest eluting

25 spots are evaporated and repurified with preparative HPLC (C18 Deltapak 30 $\times$ 300 mm, 15% to 70%  $\text{CH}_3\text{CN}$  in water).

$^1\text{H}$  nmr (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8,31 (d, 1H,  $J=7,6$  Hz), 7,39 (m, 4H), 7,26 (m, 3H), 7,16 (m, 4H), 6,31 (d, 1H,  $J=4,4$  Hz),

30 5,32 (t, 1H,  $J=2,3$  Hz), 4,69 (dd, 1H,  $J=12,6$  and 2,6 Hz), 4,52 (dd, 1H,  $J=12,6$  and 2,0 Hz), 4,38 (d, 1H,  $J=10,2$  Hz), 4,30 (m, 1H).

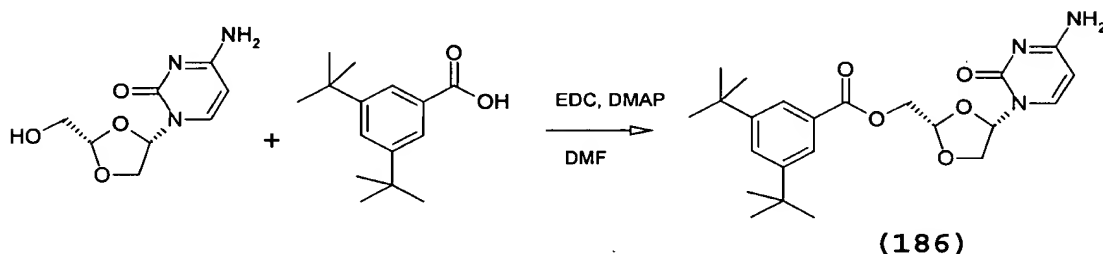
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40

5

EXAMPLE 34

**3,5-Di-tert.-butylbenzoic acid 4-(4-amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethyl ester**



10

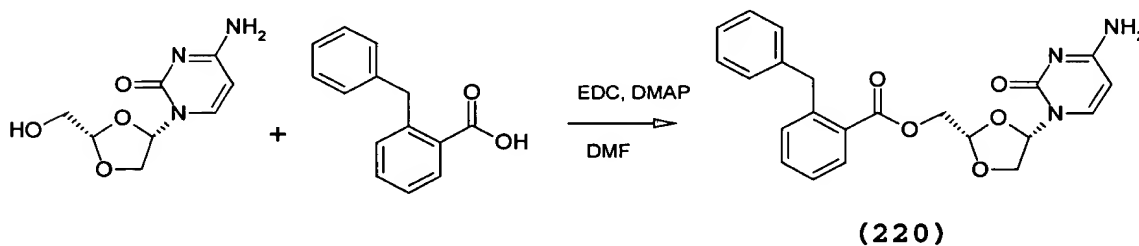
**Procedure:** The nucleoside (495 mg, 2.32 mmol, 1.0eq), 3,5-di-tButylbenzoic acid (545 mg, 2.32 mmol, 1.0eq), DMAP (30 mg, 0.23 mmol, 0.1eq) and EDC (445 mg, 2.32 mmol, 1.0eq) were mixed in DMF and stirred at room temperature. The solvent was mostly evaporated and the crude diluted in dichloromethane. The organic layer was washed twice with water, brine, dried over magnesium sulfate, filtered and evaporated to dryness. The desired compound was isolated by flash chromatography using a gradient of 3%-10% methanol in dichloromethane. 281 mg was obtained.

<sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>): 7.76 (s, 2H), 7.70 (s, 1H), 7.49 (d, J=7.5Hz, 1H), 7.18 (br d, J=24.2Hz, 2H), 6.23 (m, 1H), 5.46 (d, J=7.5Hz, 1H), 5.26 (t, J=3.3Hz, 1H), 4.55 (m, 2H), 4.15-4.05 (m, 2H), 1.28 (m, 18H).

30

EXAMPLE 35

**Preparation of 2-Benzylbenzoic acid 4-(4-amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethyl ester**



35

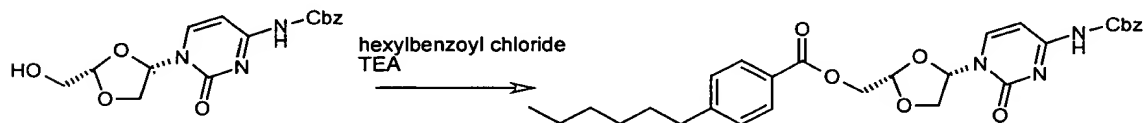
5

**Procedure:** The nucleoside (444 mg, 2.10 mmol, 1.0eq), alphaphenyl-o-toluic acid (445 mg, 2.10 mmol, 1.0eq), DMAP (27 mg, 0.21 mmol, 0.1eq) and EDC (400 mg, 2.10 mmol, 1.0eq) were mixed in DMF and stirred at room temperature. The solvent was mostly evaporated and the crude diluted in dichloromethane. The organic layer was washed twice with water, brine, dried over magnesium sulfate, filtered and evaporated to dryness. The desired compound was isolated by flash chromatography using a gradient of 3%-10% methanol in dichloromethane.

<sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>): 7.77 (m, 1H), 7.56-7.48 (m, 2H), 7.38-7.31 (m, 2H), 7.24-7.08 (m, 7H), 6.23 (m, 1H), 5.44 (d, J=7.5Hz, 1H), 5.19 (t, J=3.0Hz, 1H), 4.47 (m, 2H), 4.27 (m, 2H), 4.11 (m, 2H).

#### EXAMPLE 36

**PREPARATION OF 4-HEXYL-BENZOIC ACID 4-(4-METHYLAMINO-2-  
OXO-2H-PYRIMIDIN-1-YL) - [1,3]DIOXOLAN-2-YLMETHYL ESTER**



#### Procedure:

30

Acid chloride (64~~μ~~L, 0.29mmol, 1eq.) was added to the mixture of the Cbz-protected BCH-4556 (101mg, 0.29mmol) in CH<sub>2</sub>Cl<sub>2</sub> with TEA (0.12mL, 0.87mmol, 3eq.). Reaction mixture was stirred at room temperature for 2 days. Solvent was evaporated. Purification was done by flash chromatography using MeOH/CH<sub>2</sub>Cl<sub>2</sub> 5% to give the desired compound plus some impurities.

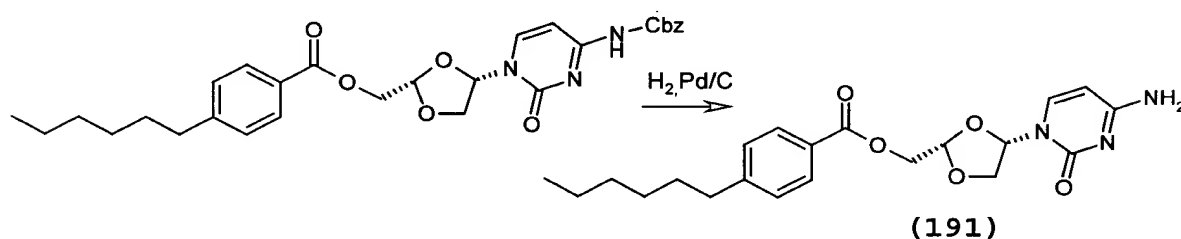
35



5  $^1\text{H}$  NMR (400MHz;  $\text{CDCl}_3$ ): 8.12 (d, 1H,  $J=7.6\text{Hz}$ ); 7.96-7.93 (m, 2H); 7.39-7.34 (m, 5H); 7.30-7.25 (m, 3H); 6.22 (dd, 1H;  $J=4.8$  and  $1.8\text{Hz}$ ); 5.34 (t, 1H,  $J=3\text{Hz}$ ); 5.21 (s, 2H); 4.77 (dd, 1H,  $J=3$  and  $12.7\text{Hz}$ ); 4.58 (dd, 1H,  $J=3$  and  $12.7\text{Hz}$ ); 4.32-4.24 (m, 2H); 2.69-2.65 (m, 2H); 1.66-1.60 (m, 2H); 1.35-1.27 (m, 6H); 0.88-0.85 (m, 3H) ppm

### EXAMPLE 37

15 **Preparation of 4-HEXYL-BENZOIC ACID 4-(4-AMINO-2-OXO-2H-PYRIMIDIN-1-YL) - [1,3]DIOXOLAN-2-YLMETHYL ESTER**



### 20 Procedure:

The protected compound (194mg, 0.29mmol) was dissolved in ethanol at  $50^\circ\text{C}$ , then purged with nitrogen. Pd/C was added, then the solution was put under  $\text{H}_2$  atmosphere and stirred at  $50^\circ\text{C}$ . The solution was filtered and  
 25 concentrated to give a foamy white solid. Purification by flash chromatography using  $\text{MeOH}/\text{CH}_2\text{Cl}_2$  3%.

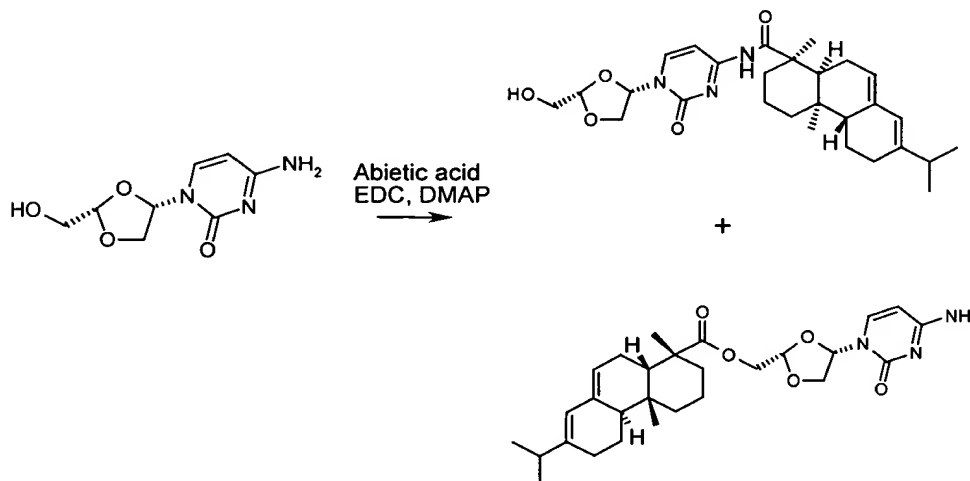
$^1\text{H}$  NMR (400MHz; DMSO): 7.87 (d, 1H,  $J=8.2\text{Hz}$ ); 7.60 (d, 1H,  $J=7.4\text{Hz}$ ); 7.37 (d, 1H,  $J=8.2\text{Hz}$ ); 6.27 (t, 1H,  $J=3.7\text{Hz}$ );  
 30 5.64 (d, 1H,  $J=7.5\text{Hz}$ ); 4.68-4.53 (m, 2H); 4.15 (d, 2H,  $J=3.9\text{Hz}$ ); 2.67 (t, 2H,  $J=7.5\text{Hz}$ ); 1.61-1.58 (m, 2H); 1.28 (m, 6H) and 0.87-0.84 (m, 3H). ppm.

### EXAMPLE 38

35

**PREPARATION OF 7-ISOPROPYL-2,4A-DIMETHYL-1,2,3,4,4A,4B,5,6,10,10A-DECAHYDRO-PHENANTHRENE-2-**

- 5 **CARBOXYLIC ACID [1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-AMIDE or ESTER**



10 **Procedure:**

- EDC (90mg, 0.47mmol) was added to a solution of the acid (143mg, 0.47mmol) and the alcohol (101mg, 0.47mmol) in DMF followed by the addition of DMAP(6mg, 0.047mmol, 0.1eq.). Reaction mixture was stirred at room temperature overnight. Reaction mixture was poured into brine, extracted with EtOAc, combined extracts were washed with NaHCO<sub>3</sub> sat. solution, dried and concentrated to give a yellow oil.
- 20 Purification by flash chromatography using MeOH/EtOAc 10% to give two compounds.

**Compound 1: amide (207)**

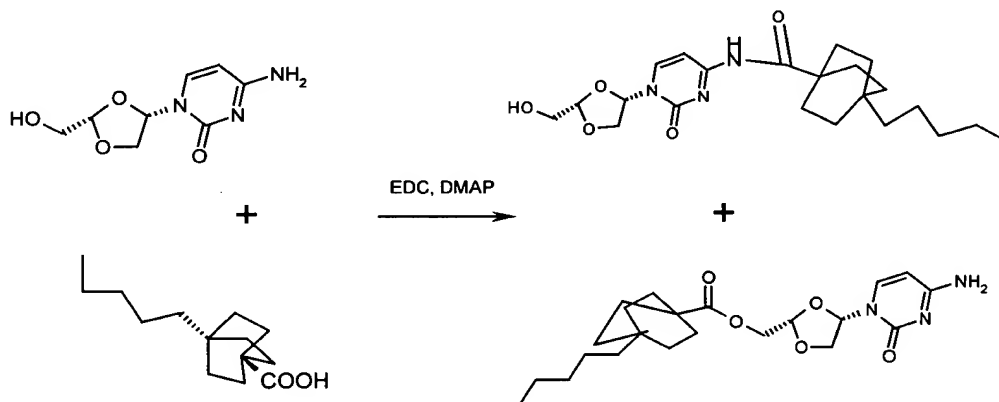
- 25 <sup>1</sup>H NMR (400MHz; CDCl<sub>3</sub>): 8.42 (d, 1H, J=7.4Hz); 8.20 (bs,NH); 7.42 (d, 1H, J=7.6Hz); 6.18 (dd, 1H, J=5.2 and 1.2Hz); 5.74 (s, 1H); 5.30 (bt, 1H); 5.12 (t, 1H, J=1.8Hz); 4.36-4.24 (m, 2H); 3.98(s, 2H); 2.63-0.85(multiplets abietic part; similar to abietic acid) ppm

5 **Compound 2: ester (281)**

H NMR (400MHz; CDCl<sub>3</sub>): 7.67 (d, 1H, J=7.5Hz); 6.19 (dd, 1H, J=2.8 and 4.5Hz); 5.71 (t, 1H, J=7.5Hz); 5.36 (d, 1H, J=3.1Hz); 5.18 (dd, 1H, J=2.1 and 4.7Hz); 4.48-4.09 (2m, 3H) and 2.24-0.83 (multiplets abietic part; similar to  
10 abietic acid) ppm

**EXAMPLE 39**

15 **PREPARATION OF 4-PENTYL-BICYCLO[2.2.2]OCTANE-1-CARBOXYLIC ACID [1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-AMIDE or ESTER**

**Procedure:**

EDC (95mg, 0.50mmol) was added to a solution of the acid  
20 (112mg, 0.50mmol) and the alcohol (106mg, 0.50mmol) in DMF  
(0.5mL) followed by the addition of DMAP (6mg, 0.050mmol,  
0.1eq.). Reaction mixture was stirred at room temperature  
overnight. Reaction mixture was poured into brine,  
extracted with EtOAc, combined extracts were washed with  
25 NaHCO<sub>3</sub> sat. solution, dried and concentrated to give a  
yellow oil.

Purification by flash chromatography using MeOH/EtOAc 10%  
to give two compounds.

30

5 **Compound 1: amide (210)**

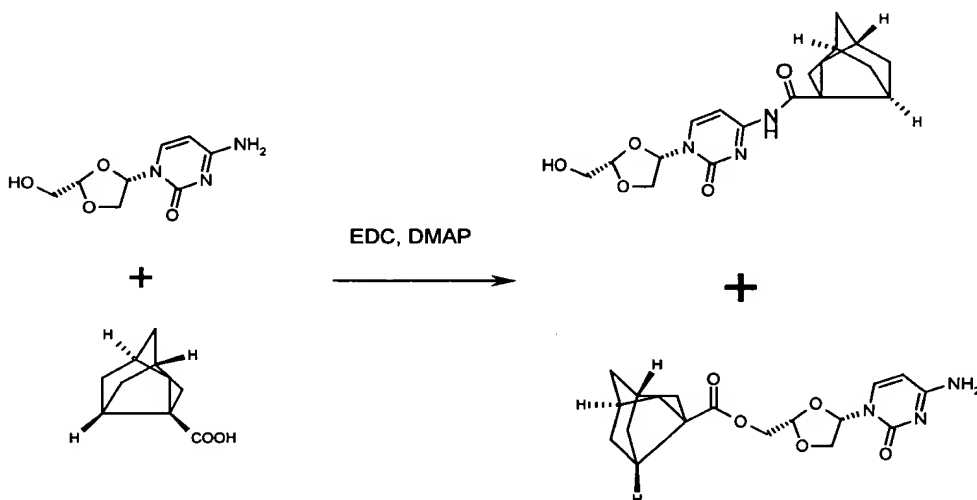
<sup>1</sup>H NMR (400MHz; CDCl<sub>3</sub>): 8.34 (d, 1H, J=7.6Hz); 7.36 (d, 1H, J= 7.6Hz); 6.11 (dd, 1H, J=5.1 and 1.3Hz); 5.06 (t, 1H, J=1.8Hz); 4.28-4.16 (m, 2H); 3.91 (d, 1H, J=1.6Hz); 1.74-1.70 (m, 6H); 1.38-1.25 (m, 6H); 1.21 0.98(m, 8H); 0.81 (t, 3H, J=7.0Hz)ppm

**Compound 2: ester (211)**

<sup>1</sup>H NMR (400MHz; CDCl<sub>3</sub>): 7.64 (d, 1H, J=7.4Hz); 6.22 (dd, 1H, J= 2.8 and 4.3Hz); 5.77 (d, 1H, J=7.5Hz); 5.15 (t, 1H, J=3.5Hz); 4.41 (dd, 2H, J= 3.7 and 12.2Hz); 4.23-4.17 (m, 1H); 1.78-1.74 (m, 6H); 1.39-1.25 (m, 6H); 1.21 1.05(m, 8H); 0.86 (t, 3H, J=7.3Hz)ppm

EXAMPLE 40

**HEXAHYDRO-2,5-METHANO-PENTALENE-3A-CARBOXYLIC ACID [1-(2-HYDROXYMETHYL-[1,3]DIOXOLAN-4-YL)-2-OXO-1,2-DIHYDRO-PYRIMIDIN-4-YL]-AMIDE or ESTER**



5 **Procedure:**

EDC (128mg, 0.67mmol) was added to a solution of the acid (111mg, 0.67mmol) and the alcohol (142mg, 0.67mmol) in DMF followed by the addition of DMAP (8mg, 0.067mmol, 0.1eq.).

10 Reaction mixture was stirred at room temperature overnight. Reaction mixture was poured into brine, extracted with EtOAc, combined extracts were washed with NaHCO<sub>3</sub> sat. solution, dried and concentrated to give a yellow oil.

15 Purification by flash chromatography using MeOH/EtOAc 5% to give two compounds.

Compound 1: amide (231)

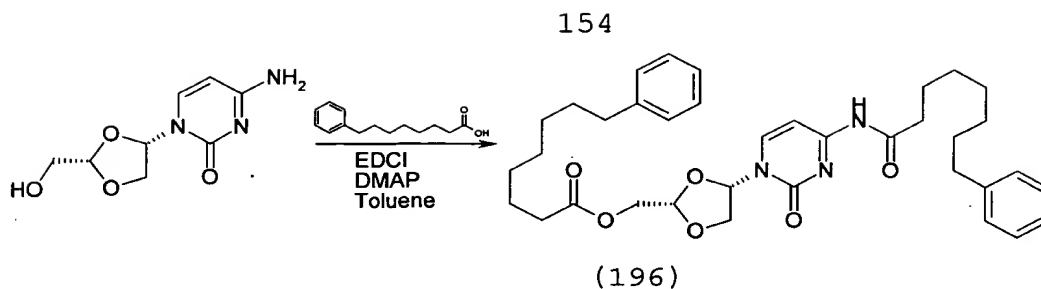
20 <sup>1</sup>H NMR (400MHz; CDCl<sub>3</sub>): 8.46 (d, 1H, J=7.5Hz); 7.98 (bs, 1H); 7.40 (d, 1H, J= 7.5Hz); 6.19 (d, 1H, J=4.9Hz); 5.12 (s, 1H); 4.33-4.21 (m, 2H); 3.98 (s, 2H); 3.28 (bs, 1H); 2.74 (t, 1H, J=6.7Hz); 2.37 (s, 1H); 2.16 (s, 2H); 2.04-  
25 2.01 (m, 2H); 1.86-1.82 (m, 4H) and 1.70-1.62 (m, 4H)ppm

Compound 2: ester (232)

H NMR (400MHz; CDCl<sub>3</sub>): 7.74 (d, 1H, J=7.4Hz); 6.25 (t, 1H, J= 3.8Hz); 5.72 (d, 1H, J=7.4Hz); 5.23 (t, 1H, J=3.6Hz);  
30 4.55-4.29 (m, 2H); 4.24 (d, 2H, J=3.7Hz); 2.72-2.71 (m, 1H); 2.33 (m, 2H); 2.11-2.08 (m, 2H); 1.85-1.82 (m, 4H) and 1.68-1.61 (m, 4H)ppm

**EXAMPLE 41**

35 **Preparation of 8-Phenyl-octanoic acid 4-[2-oxo-4-(8-phenyl-octanoylamino)-2H-pyrimidin-1-yl]-[1,3]dioxolan-2-ylmethyl ester**



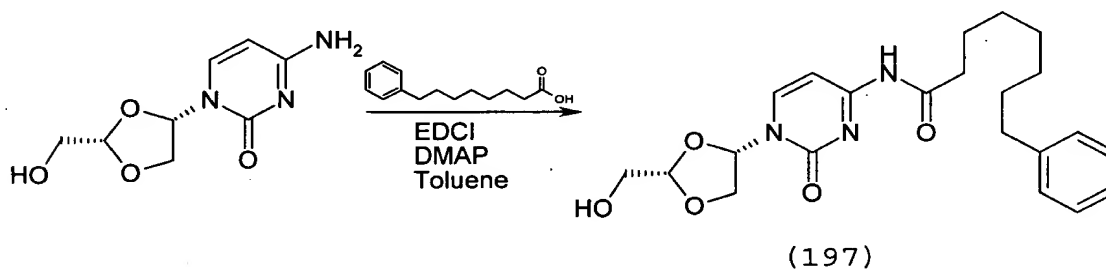
**Procedure:**

4-Amino-1-(2-hydroxymethyl-[1,3]dioxolan-4-yl)-1H-pyrimidin-2-one (0.23 mmol) was treated with 8-phenyl-octanoic acid (0.23 mmol), EDCI (0.35 mmol) and DMAP (catalytic amount) in DMF for 14 hours. The solution was neutralized with NaHCO<sub>3</sub> sat. and extracted with AcOEt. The combined organic layers were dried over sodium sulfate, filtered and concentrated in vacuum. The residue was purified by bond elute (2% MeOH/CH<sub>2</sub>Cl<sub>2</sub> to 10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to afford 8-Phenyl-octanoic acid 4-[2-oxo-4-(8-phenyl-octanoylamino)-2H-pyrimidin-1-yl]-[1,3]dioxolan-2-ylmethyl ester.

<sup>1</sup>H NMR (CDCl<sub>3</sub>) 8.70 (s, 1H), 8.15 (d, J = 7.5 Hz, 1H), 7.50 (d, J = 7.4 Hz, 1H), 7.30-7.17 (m, 10H), 6.22 (d, J = 4.7 Hz, 1H), 5.24 (t, J = 2.6 Hz, 1H), 4.58 (dd, J = 12.6, 2.8 Hz, 1H), 4.32-4.25 (m, 3H), 2.63-2.59 (m, 4H), 2.48-2.36 (m, 4H), 1.80-1.60 (m, 8H), 1.45-1.25 (m, 12H).

**EXAMPLE 42**

**8-Phenyl-octanoic acid [1-(2-hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-amide**



5 **Procedure:**

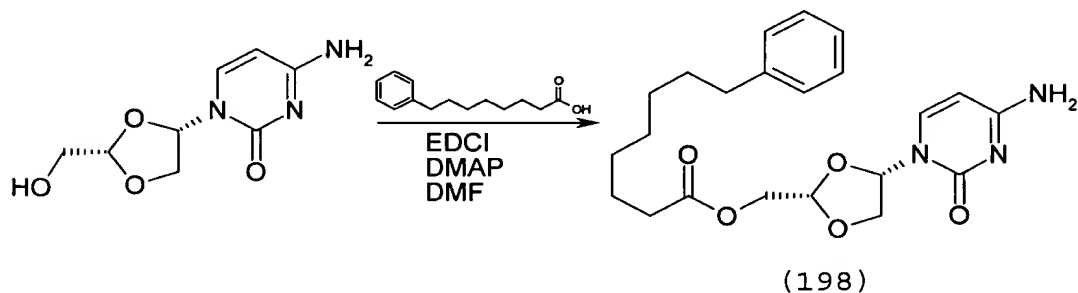
4-Amino-1-(2-hydroxymethyl-[1,3]dioxolan-4-yl)-1H-pyrimidin-2-one (0.23 mmol) was treated with 8-Phenyl-octanoic acid (0.23 mmol), EDCI (0.35 mmol) and DMAP (catalytic amount) in DMF for 14 hours. The solution was neutralized with NaHCO<sub>3</sub> sat. and extracted with AcOEt. The combined organic layers were dried over sodium sulfate, filtered and concentrated in vacuum. The residue was purified by bond elute (2% MeOH/CH<sub>2</sub>Cl<sub>2</sub> to 10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to produce 8-Phenyl-octanoic acid [1-(2-hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-amide.

HNMR (CDCl<sub>3</sub>) 8.62 (s, 1H), 8.49 (d, J= 7.5 Hz, 1H), 7.45 (d, J= 7.5 Hz, 1H), 7.30-7.27 (m, 2H), 7.20-7.17 (m, 3H), 6.20 (d, J= 4.5 Hz, 1H), 5.14 (s, 1H), 4.33-4.26 (m, 2H), 3.98 (s, 2H), 2.60 (t, J= 7.6 Hz, 2H), 2.45 (t, J= 7.5 Hz, 2H), 1.68-1.60 (m, 4H), 1.40-1.30 (m, 6H).

25 **EXAMPLE 43**

**8-Phenyl-octanoic acid 4-(4-amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethyl ester**

30

**Procedure:**

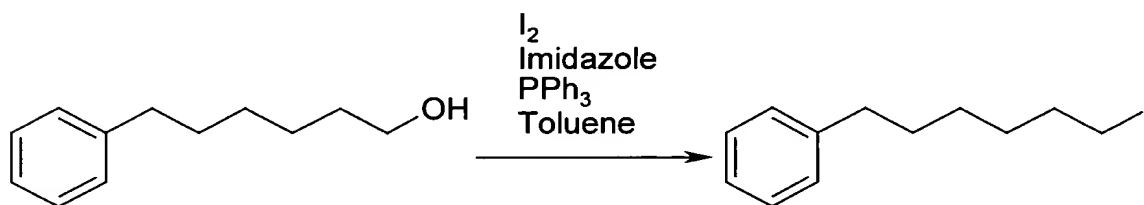
35 4-Amino-1-(2-hydroxymethyl-[1,3]dioxolan-4-yl)-1H-pyrimidin-2-one (0.23 mmol) was treated with 8-phenyl-

5 octanoic acid (0.23 mmol), EDCI (0.35 mmol) and DMAP  
 (catalytic amount) in DMF for 14 hours. The solution was  
 neutralized with NaHCO<sub>3</sub> sat. (20 mL) and extracted with  
 AcOEt. The combined organic layers were dried over sodium  
 sulfate, filtered and concentrated in vacuum. The residue  
 10 was purified by bond elute (2% MeOH/CH<sub>2</sub>Cl<sub>2</sub> to 10%  
 MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to afford 0.015g (16%) of 8-phenyl-octanoic  
 acid 4-(4-amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-  
 ylmethyl ester.

15 HNMR (CDCl<sub>3</sub>) 9.4 (s, 1H), 7.71 (d, J= 7.5 Hz, 1H), 7.51-  
 7.06 (m, 5H), 6.26 (dd, J= 5, 2 Hz, 1H), 5.78 (d, J= 7.5  
 Hz, 1H), 5.19 (t, J= 3.2 Hz, 1H), 4.48 (dd, J= 12.3, 3.3  
 Hz, 1H), 4.39-4.07 (m, 3H), 2.61 (t, J= 7.2 Hz, 2H), 2.36  
 (t, J= 7.4 Hz, 2H), 1.77-1.50 (m, 4H), 1.49-1.06 (m, 6H).

20

25

**EXAMPLE 44****(6-Iodo-hexyl) -benzene**

30

**Procedure:**

In a solution of 6-phenyl-hexan-1-ol (5.54 mmol) in  
 35 toluene (0.2 M) was added in order PPh<sub>3</sub> (12.1 mmol),  
 imidazole (24.9 mmol) and I<sub>2</sub> (11.6 mmol). The solution was  
 mixed to reflux for 1.5 h and was cooled to room

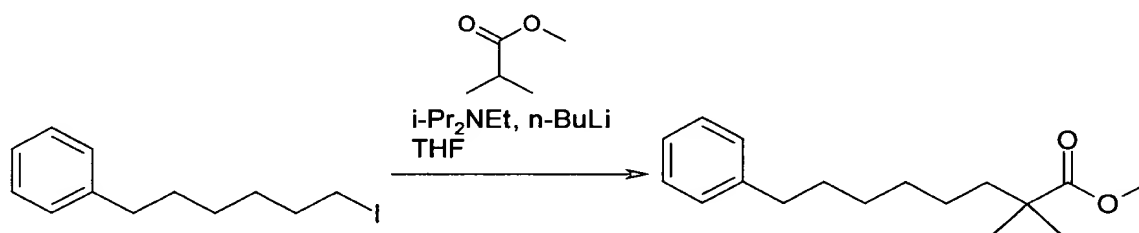


temperature. The solution was dissolved in Et<sub>2</sub>O and washed with H<sub>2</sub>O and brine. The organic layer was dried over sodium sulfate, filtered and concentrated in vacuum. The residue was purified by biotage (100% pentane to 5% Et<sub>2</sub>O/pentane) to produce (6-iodo-hexyl)-benzene.

<sup>1</sup>H NMR (CDCl<sub>3</sub>) 7.68-7.14 (m, 5H), 3.18 (t, J = 7 Hz, 2H), 2.61 (t, J = 7.6 Hz, 2H), 1.86-1.79 (m, 2H), 1.67-1.60 (m, 2H), 1.46-1.33 (m, 4H).

#### EXAMPLE 45

#### 2,2-Dimethyl-8-phenyl-octanoic acid methyl ester



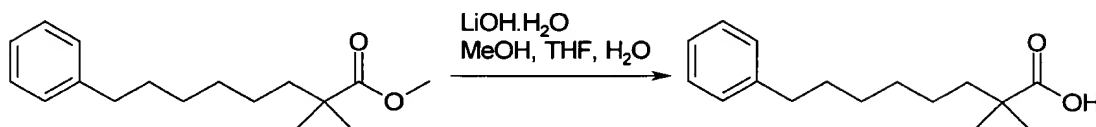
#### Procedure:

To a solution of i-Pr<sub>2</sub>NEt (2.12 mmol) in THF (0.2 M) was added a solution of 1.4 M n-BuLi in hexane (2.12 mmol) at 0°C. The mixture was stirred at 0°C for 30 minutes and cooled to -78°C for addition of isobutyric acid methyl ester (2.12 mmol). Then, the solution was stirred at -78°C for 1 hour and (6-Iodo-hexyl)-benzene (1.92 mmol) dissolved in THF was added slowly. This mixture was stirred 1 hour at -78°C and 3 hours at room temperature. The solution was dissolved in Et<sub>2</sub>O and washed with NH<sub>4</sub>Cl sat. and brine. The organic layer was dried over sodium sulfate, filtered and concentrated in vacuum. The residue was purified by bond elute (3% Et<sub>2</sub>O/pentane) to afford 0.45g (90%) of 2,2-dimethyl-8-phenyl-octanoic acid methyl ester.

HNMR (CDCl<sub>3</sub>) 7.29-7.25 (m, 2H), 7.18-7.15 (m, 3H), 3.64 (s, 3H), 3.48 (q, J= 7 Hz, 2H), 2.58 (t, J= 7.6 Hz, 2H), 1.59-1.47 (m, 2H), 1.32-1.25 (m, 2H), 1.20-1.14 (m, 10H).

#### EXAMPLE 46

##### 2,2-Dimethyl-8-phenyl-octanoic acid



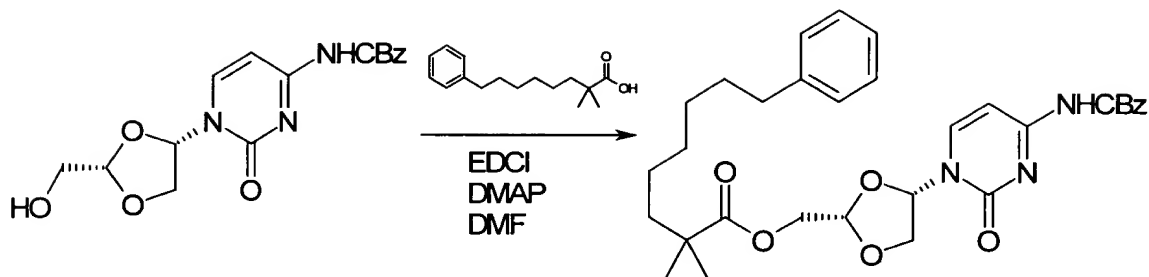
#### Procedure:

2,2-Dimethyl-8-phenyl-octanoic acid methyl ester (1.7 mmol) was dissolved in a MeOH, THF, H<sub>2</sub>O solution (10:5:2). LiOH monohydrate was added and the solution was stirred and refluxed for 7 hours. The mixture was diluted with AcOEt and extracted with a solution of saturated NaHCO<sub>3</sub>. The aqueous layers was combined, acidified with HCl 1 N and extracted with AcOEt. The organic layer was dried over sodium sulfate, filtered and concentrated in vacuum to afford 2,2-dimethyl-8-phenyl-octanoic acid.

HNMR (CDCl<sub>3</sub>) 7.23-7.18 (m, 2H), 7.12-7.08 (m, 3H), 2.52 (t, J= 7.9 Hz, 2H), 1.55-1.43 (m, 4H), 1.26-1.18 (m, 6H), 1.11 (s, 6H).

#### EXAMPLE 47

2,2-Dimethyl-8-phenyl-octanoic acid 4-(4-benzyloxycarbonylamino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethyl ester



5

**Procedure:**

10 [1-(2-Hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-carbamic acid benzyl ester (0.058 mmol) was treated with 2,2-dimethyl-8-phenyl-octanoic acid (0.058 mmol), EDCI (0.087 mmol) and DMAP (catalytic amount) in DMF. The solution was diluted in AcOEt and washed with NaHCO<sub>3</sub> sat. and brine. The organic layer was

15 dried over sodium sulfate, filtered and concentrated in vacuum. The residue was purified by bond elute (5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to afford 2,2-Dimethyl-8-phenyl-octanoic acid 4-(4-benzyloxycarbonylamino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethyl ester.

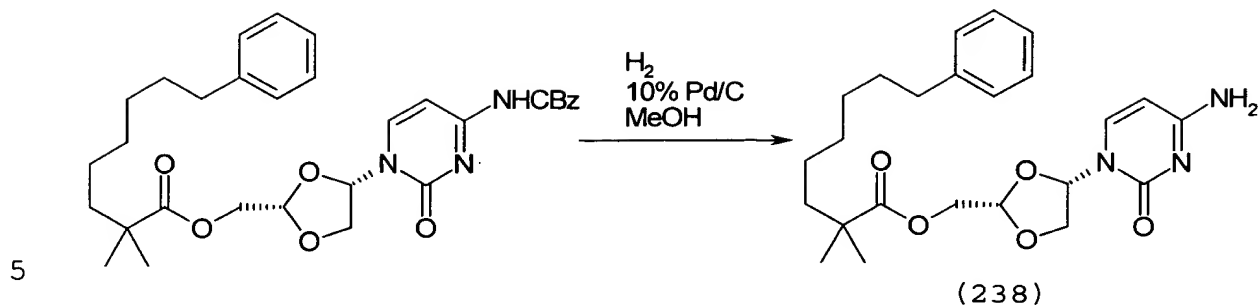
20

HNMR (MeOD) 8.20 (d, J= 7.5 Hz, 1H), 7.44-7.34 (m, 5H), 7.27-7.10 (m, 7H), 6.19 (t, J= 3.6 Hz, 1H), 5.27 (t, J= 3.2 Hz, 1H), 5.23 (s, 2H), 4.70-4.47 (m, 2H), 4.31-4.23 (m, 2H), 2.62-2.54 (m, 2H), 1.63-1.49 (m, 4H), 1.39-1.15

25 (m, 12H).

**EXAMPLE 48**

**2,2-Dimethyl-8-phenyl-octanoic acid 4-(4-amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethyl ester**



**Procedure:**

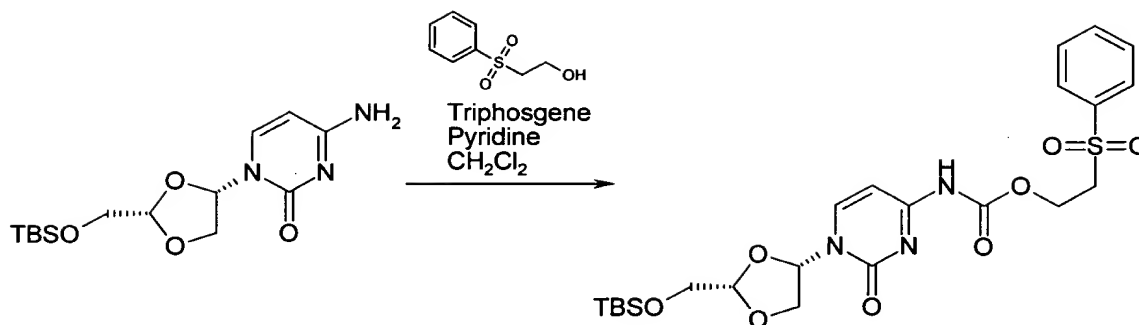
2,2-Dimethyl-8-phenyl-octanoic acid 4-(4-  
 10 benzyloxycarbonylamino-2-oxo-2H-pyrimidin-1-yl)-  
 [1,3]dioxolan-2-ylmethyl ester (0.048 mmol) was dissolved  
 in MeOH. 10% Pd/C (30% w/w) was added and the solution  
 was mixed under H<sub>2</sub>. The solution was filtered on celite  
 and concentrated in vacuum. The residue was purified by  
 15 bond elute (5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to afford of 2,2-dimethyl-8-  
 phenyl-octanoic acid 4-(4-amino-2-oxo-2H-pyrimidin-1-yl)-  
 [1,3]dioxolan-2-ylmethyl ester.

HNMR (MeOD) 7.76 (d, J= 7.5 Hz, 1H), 7.24-7.20 (m, 2H),  
 20 7.14-7.11 (m, 3H), 6.20 (dd, J= 4.5, 2.9 Hz, 1H), 5.91 (d,  
 J= 7.5 Hz, 1H), 5.18 (t, J= 3.4 Hz, 1H), 4.46 (dd, J=  
 12.4, 3.5 Hz, 1H), 4.24 (dd, J= 12.4, 3.2 Hz, 1H), 4.14  
 (t, J= 2.5 Hz, 2H), 2.56 (t, J= 7.6 Hz, 2H), 1.56-1.48 (m,  
 4H), 1.28-1.22 (m, 6H), 1.17 (s, 3H), 1.16 (s, 3H).

25

EXAMPLE 49

{1-[2-(*tert*-Butyl-dimethyl-silanyloxymethyl)-  
 [1,3]dioxolan-4-yl]-2-oxo-1,2-dihydro-pyrimidin-4-yl}-  
 30 carbamic acid 2-benzenesulfonyl-ethyl ester



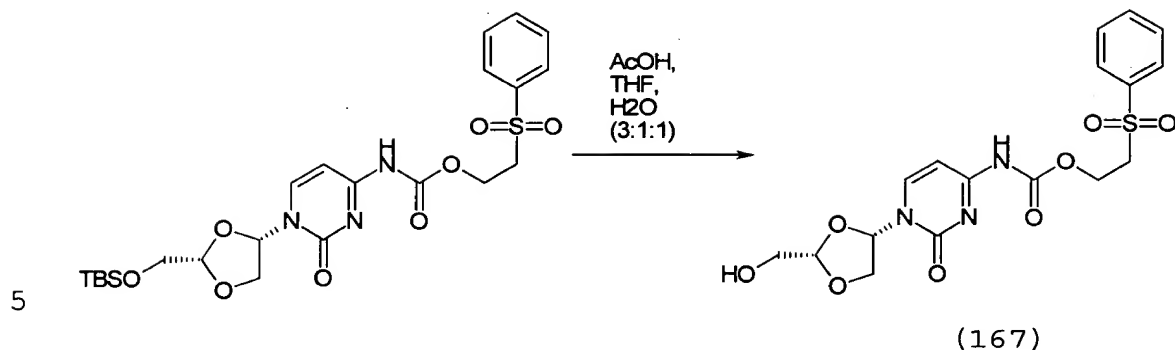
### Procedure:

To a solution of triphosgene and 2-benzenesulfonyl-ethanol in CH<sub>2</sub>Cl<sub>2</sub> was added pyridine at 0°C. This solution was mixed at 0°C added to a solution of 4-amino-1-[2-(tert-butyl-dimethyl-silanyloxymethyl)-[1,3]dioxolan-4-yl]-1H-pyrimidin-2-one and pyridine in CH<sub>2</sub>Cl<sub>2</sub>. The resulting solution was mixed and diluted in CH<sub>2</sub>Cl<sub>2</sub>. The mixture was washed with water and the organic layer was dried over sodium sulfate, filtered and concentrated in vacuo. The residue was purified by bond elute (3% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to afford {1-[2-(tert-butyl-dimethyl-silanyloxymethyl)-[1,3]dioxolan-4-yl]-2-oxo-1,2-dihydro-pyrimidin-4-yl}-carbamic acid 2-benzenesulfonyl-ethyl ester.

<sup>1</sup>H NMR (CDCl<sub>3</sub>) 8.36 (d, J = 7.2 Hz, 1H), 7.84-7.80 (m, 2H), 7.62-7.45 (m, 4H), 6.98 (s, 1H), 6.10 (dd, J = 4.7, 1.9 Hz, 1H), 4.94 (t, J = 1.9 Hz, 1H), 4.43 (t, J = 5.4 Hz, 2H), 4.16-4.08 (m, 2H), 3.93-3.84 (m, 2H), 3.46-3.42 (m, 2H), 0.82 (s, 9H), 0.02 (s, 3H), 0.00 (s, 3H).

### EXAMPLE 50

[1-(2-Hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-carbamic acid 2-benzenesulfonyl-ethyl ester



# Procedure:

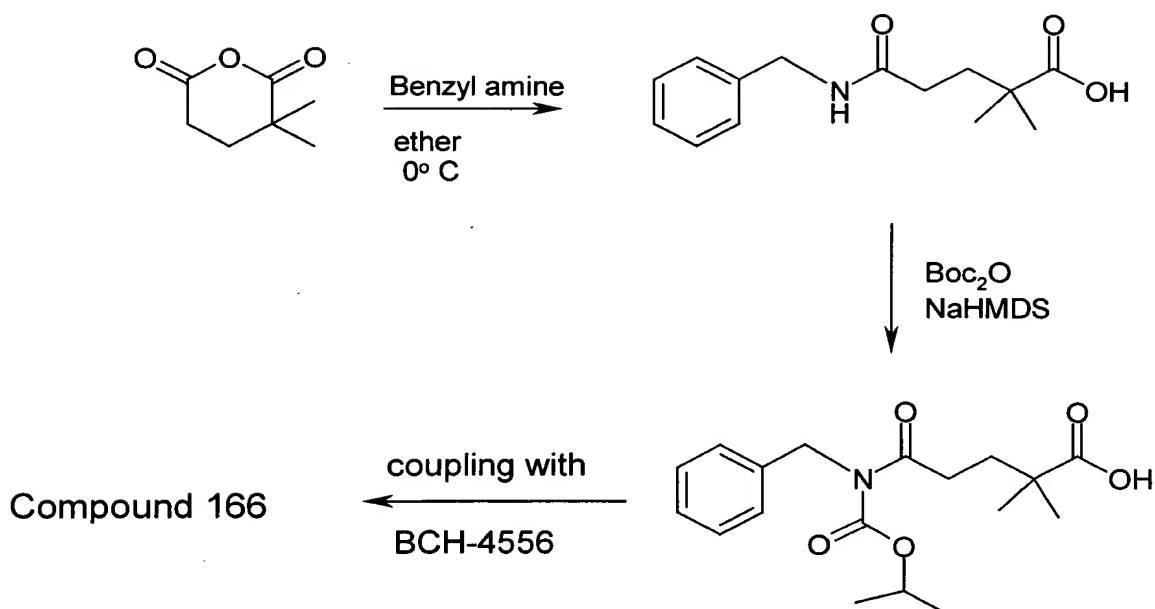
{1-[2-(*tert*-Butyl-dimethyl-silanyloxymethyl)-  
 10 [1,3]dioxolan-4-yl]-2-oxo-1,2-dihydro-pyrimidin-4-yl}-  
 carbamic acid 2-benzenesulfonyl-ethyl ester (0.087mmol)  
 was dissolved in a solution of AcOH, THF, H<sub>2</sub>O (3:1:1) and  
 was mixed. The mixture was dissolved in AcOEt and washed  
 with H<sub>2</sub>O, brine. The organic layer was dried over sodium  
 15 sulfate, filtered and concentrated in vacuo. The residue  
 was purified by bond elute (5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to afford [1-  
 (2-Hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-  
 pyrimidin-4-yl]-carbamic acid 2-benzenesulfonyl-ethyl  
 ester.

20 HNMR (CDCl<sub>3</sub>) 8.45 (d, J= 7.5 Hz, 1H), 7.93-7.90 (m, 2H),  
 7.70-7.65 (m, 2H), 7.59-7.55 (m, 2H), 7.08 (s, 1H), 6.17  
 (dd, J= 5.1, 1.2 Hz, 1H), 5.12 (t, J= 1.6 Hz, 1H), 4.53  
 (d, J= 5.9 Hz, 2H), 4.33 (dd, J= 10.6, 1.3 Hz, 1H), 4.23  
 25 (dd, J= 10.2, 5.1 Hz, 1H), 3.97 (s, 2H), 3.54-3.51 (m,  
 2H), 2.6 (s, 1H).

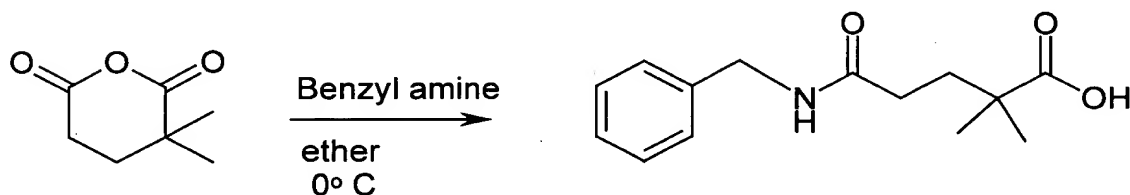
## EXAMPLE 51

30

5-(Benzyl-*tert*-butoxycarbonyl-amino)-2,2-dimethyl-5-oxo-  
 pentanoic acid



A) 4-Benzylcarbamoyl-2,2-dimethyl-butyric acid

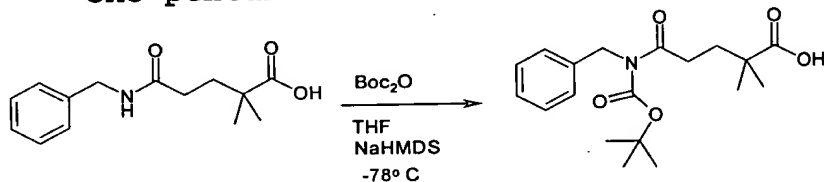


Procedure:

To a solution of 3,3-dimethyl-dihydro-pyran-2,6-dione (1.76 mmole) in diethyl ether at 0° C was added benzyl amine (1.76 mmole) dropwise. As soon as addition was made, solid started to separate. The mixture was stirred at 0° C for 15 minutes. It was diluted with ether. The solution was washed with 0.1 N HCl, and with saturated sodium chloride solution and dried over sodium sulfate. The crude product obtained after removing the solvent was passed through a bond-elute (elutents:  $\text{CH}_2\text{Cl}_2$ , 2 and 4 % MeOH in  $\text{CH}_2\text{Cl}_2$ ) yielding 4-benzylcarbamoyl-2,2-dimethyl-butyric acid (57%).

HNMR ( $\delta$ , CD<sub>3</sub>OD): 7.23-7.32 (5H, m), 4.34 (2H, s), 2.21-2.26 (2H, m), 1.83-1.87 (2H, m), 1.18 (6H, s).

**B) 5-(Benzyl-tert-butoxycarbonyl-amino)-2,2-dimethyl-5-oxo-pentanoic acid**



**Procedure:**

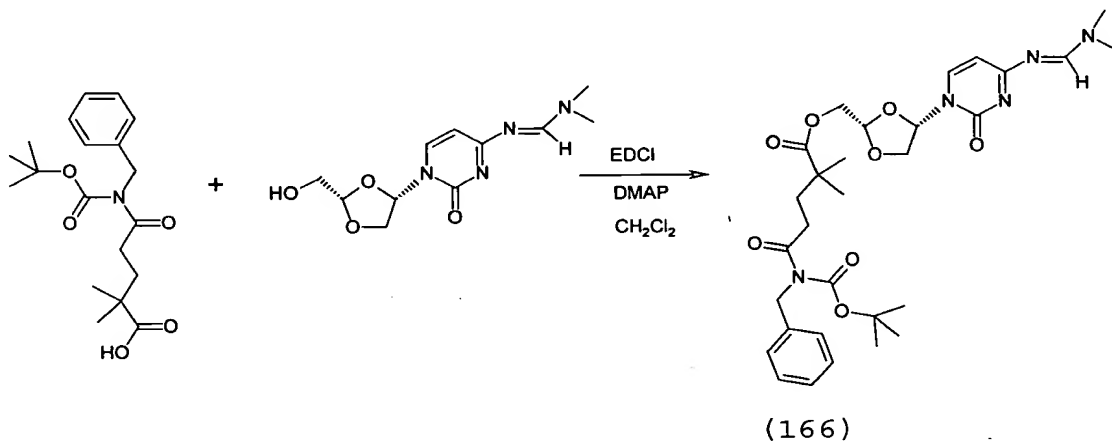
To a solution of 4-benzylcarbamoyl-2,2-dimethyl-butyr-  
ic acid (0.09 mmole) in THF at -78° C was added NaHMDS in THF  
(1M) dropwise. It was stirred at -78° C for 15 minutes. Di-  
tert-butyl dicarbonate (0.1 mmole) in THF was added. It  
was stirred at this temperature for 15 minutes. Saturated  
NH<sub>4</sub>Cl solution was added and the mixture was allowed to  
come to room temperature. It was acidified with dil. HCl  
and extracted with ethyl acetate. The extract was washed  
with saturated sodium chloride solution and dried over  
sodium sulfate. The solvent was removed and the residue  
was passed through a bond-elute (elutents: CH<sub>2</sub>Cl<sub>2</sub> and 5%  
MeOH in CH<sub>2</sub>Cl<sub>2</sub>) yielding 5-(benzyl-tert-butoxycarbonyl-  
amino)-2,2-dimethyl-5-oxo-pentanoic acid (39%).

HNMR ( $\delta$ , CDCl<sub>3</sub>): 7.22-7.31 (5H, m), 4.87 (2H, s), 2.91-2.95  
(2H, m), 1.93-1.97 (2H, m), 1.40 (9H, s), 1.24 (6H, s).

**EXAMPLE 52**

**5-(Benzyl-tert-butoxycarbonyl-amino)-2,2-dimethyl-5-oxo-  
pentanoic acid 4-[4-(dimethylamino-methyleneamino)-2-oxo-  
2H-pyrimidin-1-yl]-[1,3]dioxolan-2-ylmethyl ester**





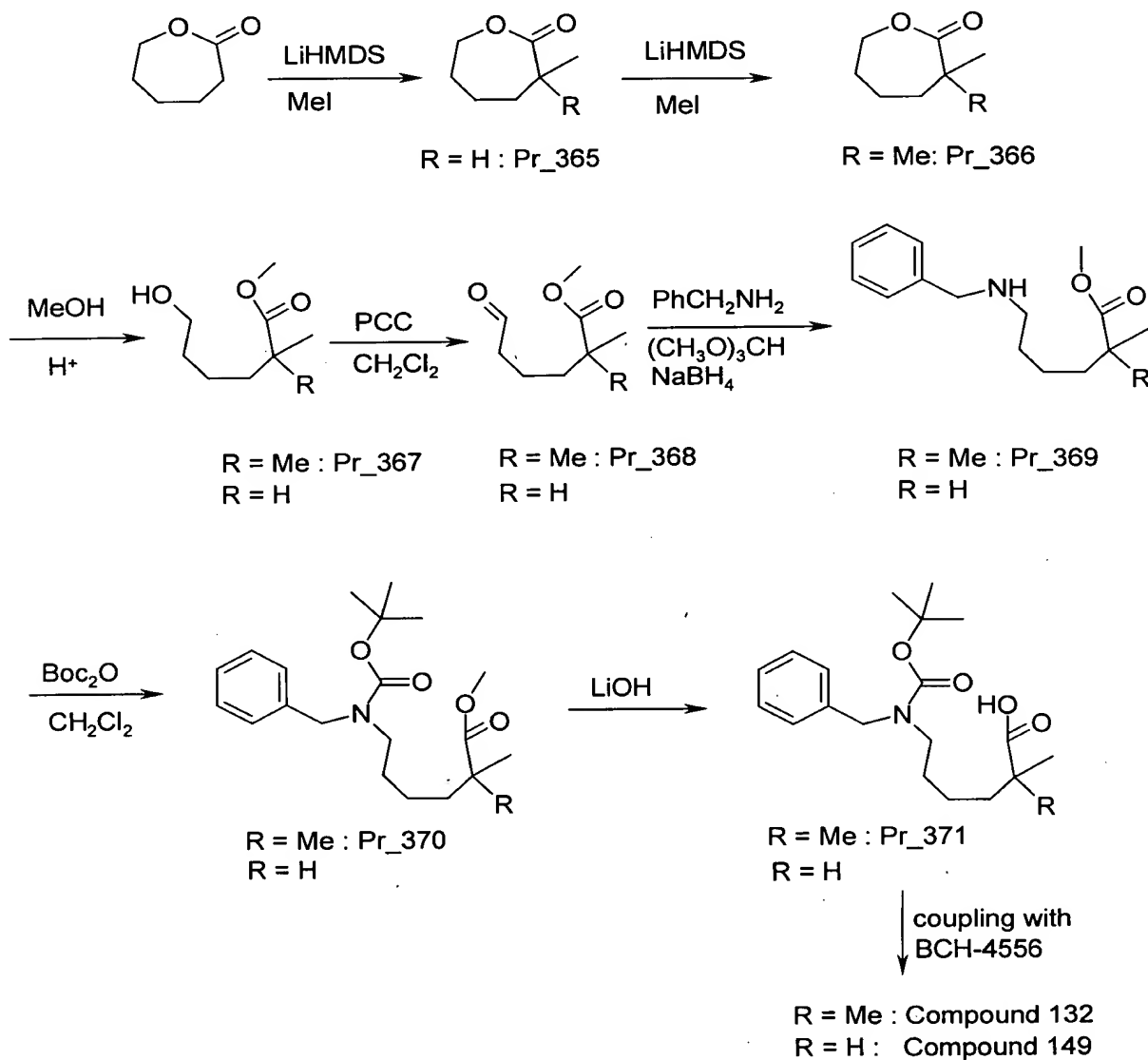
### Procedure:

To a solution of N'-[1-(2-hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-N,N-dimethylformamide (0.034 mmole), 5-(benzyl-tert-butoxycarbonylamino)-2,2-dimethyl-5-oxopentanoic acid (0.034 mmole) and DMAP in CH<sub>2</sub>Cl<sub>2</sub> at 0° C was added EDCI (0.078 mmole) in CH<sub>2</sub>Cl<sub>2</sub> dropwise. The mixture was stirred at 0° C for 0.5 hr and then at room temperature for 18 hrs. It was diluted with CH<sub>2</sub>Cl<sub>2</sub>, washed with water and saturated sodium chloride solution. The solution was dried over sodium sulfate and the solvent was evaporated. The pure ester was obtained after flash chromatography over bond-elute (elutents: CH<sub>2</sub>Cl<sub>2</sub>, 2 and 4 % MeOH in CH<sub>2</sub>Cl<sub>2</sub>) in 44% yield.

<sup>1</sup>H NMR (δ, CD<sub>3</sub>OD): 8.67 (1H, s), 7.97 (1H, d, J = 7.2 Hz), 7.16-7.30 (5H, m), 6.20 (1H, d, J = 7.2 Hz), 6.17 (1H, t, J = 3.7 Hz), 5.25 (1H, dd, J = 2.9, 3.4 Hz), 4.83 (2H, fine split signal), 4.57 (1H, dd, J = 3.5, 12.6 Hz), 4.27 (1H, dd, J = 2.9, 12.5 Hz), 4.21 (2H, d, J = 3.7 Hz), 3.21, 3.13 (3H each, fine split singlets), 2.86-2.92 (2H, m), 1.89-1.93 (2H, m), 1.36 (9H, s), 1.24, 1.22 (3H each, s).

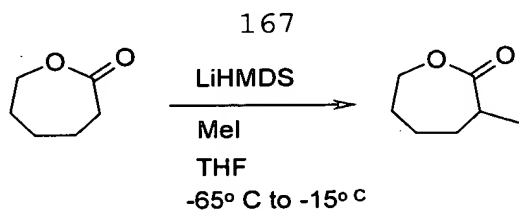
5 EXAMPLE 53

6-(Benzyl-*tert*-butoxycarbonyl-amino)-2,2-dimethyl-hexanoic acid and 6-(benzyl-*tert*-butoxycarbonyl-amino)-2-methyl-hexanoic acid



10

## A) 3-Methyl-oxepan-2-one



5

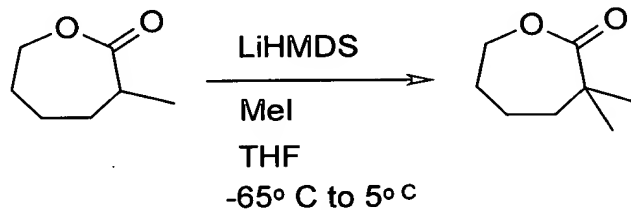
**Procedure:**

- 10 A solution of oxepan-2-one (4.54 mmole) in THF cooled to -65°C was treated with LiHMDS (1M). The mixture was stirred at -65°C. Methyl iodide (8.03 mmole) was added. The temperature was raised slowly to -15°C. Saturated NH<sub>4</sub>Cl solution was added. The mixture was extracted with diethyl
- 15 ether. The solution was dried over sodium sulfate and the solvent was evaporated. The crude was passed through a bond-elute (eluent: pentane-ether mixture - 1:1) yielding 3-methyl-oxepan-2-one contaminated with small amount of 3,3-dimethyl-oxepan-2-one (about 13% from NMR) (around 52
- 20 %).

HNMR ( $\delta$ , CDCl<sub>3</sub>): 4.20-4.34 (2H, m), 2.71-2.76 (1H, m), 1.93-2.01 (2H, m), 1.52-1.76 (4H, m), 1.23 (3H, d, J = 6.7 Hz)

25

**B) 3,3-Dimethyl-oxepan-2-one**



30

**Procedure:**

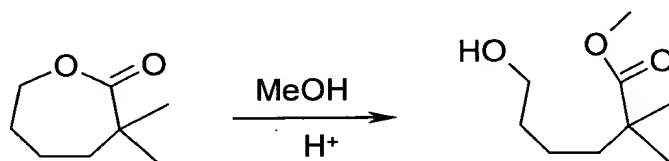
5 A solution of 3-methyl-oxepan-2-one (containing 13% of  
3,3-dimethyl-oxepan-2-one) in THF at  $-65^{\circ}\text{C}$  was treated with  
LiHMDS (1M) dropwise. The mixture was stirred at  $-65^{\circ}\text{C}$  and  
methyl iodide (28.6 mmole) was added. The temperature was  
slowly raised to  $5^{\circ}\text{C}$ . It was stirred at  $5^{\circ}\text{C}$  and saturated  
10  $\text{NH}_4\text{Cl}$  solution was added. The mixture was extracted with  
diethyl ether. The extracts were dried over sodium sulfate  
and the solvent was removed. The crude on passing through  
a bond-elute (eluent: pentane-ether-1:1) gave pure 3,3-  
dimethyl-oxepan-2-one (approx. 26%).

15

$^1\text{H NMR}$  ( $\delta$ ,  $\text{CDCl}_3$ ): 4.24-4.27 (2H, m), 1.71-1.79 (4H, m),  
1.55-1.58 (2H, m), 1.25 (6H, s).

**C) 6-Hydroxy-2,2-dimethyl-hexanoic acid methyl ester**

20

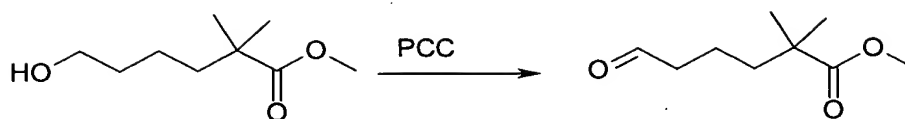


**Procedure:**

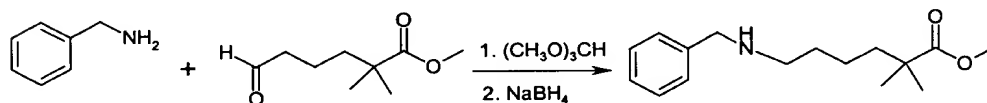
25 Methanolic HCl was prepared by adding acetyl chloride to  
dry MeOH slowly. 3,3-Dimethyl-oxepan-2-one (0.7 mmole) was  
treated with this solution. The mixture was stirred at  
room temperature. The solvent was removed. The residue was  
dissolved in diethyl ether. The solution was washed with  
30  $\text{NaHCO}_3$  solution and saturated sodium chloride solution and  
dried over sodium sulfate. The solvent was removed. The  
crude product was pure enough for the next step.

**D) 2,2-Dimethyl-6-oxo-hexanoic acid methyl ester**

35

**Procedure:**

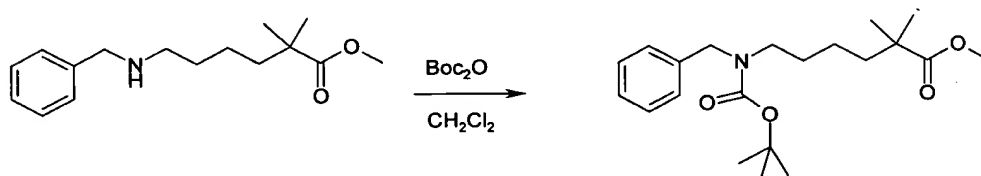
A mixture of 6-hydroxy-2,2-dimethyl-hexanoic acid methyl ester, molecular sieves 4A° and PCC in CH<sub>2</sub>Cl<sub>2</sub> was stirred at 0°C for 1 hr. It was diluted with diethyl ether and filtered through a bed of silica gel. The solvent was removed from the filtrate. The crude aldehyde thus obtained was pure enough for the next step.

**E) 6-Benzylamino-2,2-dimethyl-hexanoic acid methyl ester****Procedure:**

A mixture of benzyl amine (0.38 mmole) and methyl orthoformate (7.3 mmole) was stirred at room temperature for 5 minutes. This solution was added to crude 2,2-dimethyl-6-oxo-hexanoic acid methyl ester (0.33 mmole). It was stirred for 6 hrs. and evaporated to dryness. The residue was dissolved in MeOH and the solution was cooled to 0° C. Sodium borohydride was added in portions and the mixture was stirred. MeOH was removed and the residue was taken up in ethyl acetate. The solution was washed with saturated sodium chloride solution, dried and evaporated. The crude was passed through a bond-elute (eluent: CH<sub>2</sub>Cl<sub>2</sub>, and 1 and 2% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) yielding pure 6-benzylamino-2,2-dimethyl-hexanoic acid methyl ester (13% in three steps)

HNMR ( $\delta$ ,  $\text{CDCl}_3$ ): 7.24-7.33 (5H, m), 3.78 (2H, s), 3.64 (3H, s), 2.61 (2H, t,  $J = 7.2$  Hz), 1.45-1.53 (4H, m), 1.21-1.26 (2H, m), 1.15 (6H, s).

**F) 6-(Benzyl-tert-butoxycarbonyl-amino)-2,2-dimethyl-hexanoic acid methyl ester**

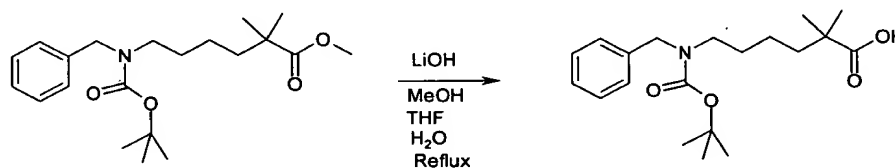


**Procedure:**

To a solution of 6-benzylamino-2,2-dimethyl-hexanoic acid methyl ester (0.09 mmole) in  $\text{CH}_2\text{Cl}_2$  (3 ml) at  $0^\circ\text{C}$  was added di-tert-butyl dicarbonate (0.14 mmole) in  $\text{CH}_2\text{Cl}_2$ . The mixture was stirred at room temperature for 2 hrs. It was evaporated to dryness and passed through a bond-elute yielding pure 6-(benzyl-tert-butoxycarbonyl-amino)-2,2-dimethyl-hexanoic acid methyl ester (85%).

HNMR ( $\delta$ ,  $\text{CDCl}_3$ ): 7.21-7.33 (5H, m), 4.39-4.42 (2H, two broad signals), 3.63 (3H, s), 3.10-3.19 (2H, broad signal), 1.43-1.48 (13H, two broad signals), 1.13 (8H, broad singlet).

**G) 6-(Benzyl-tert-butoxycarbonyl-amino)-2,2-dimethyl-hexanoic acid**



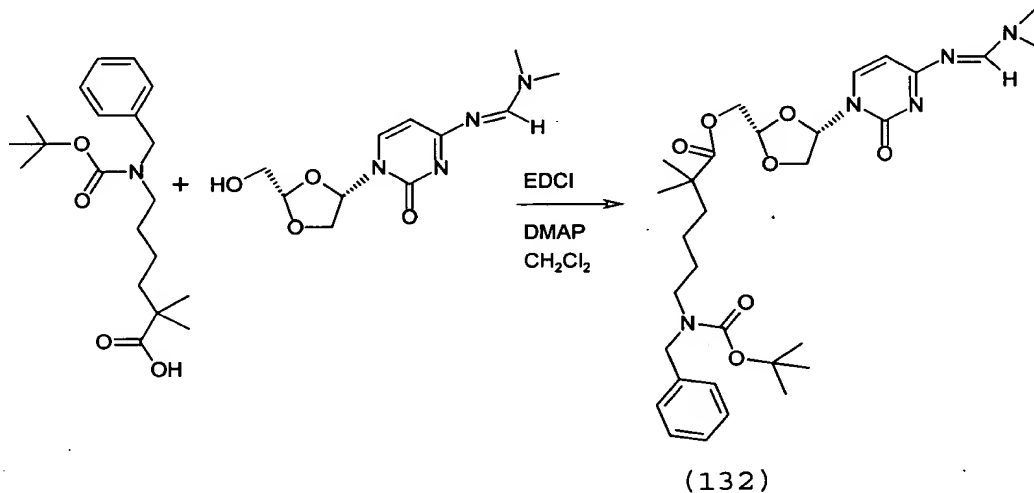
5 **Procedure:**

To a solution of 6-(benzyl-*tert*-butoxycarbonyl-amino)-2,2-dimethyl-hexanoic acid methyl ester (0.06 mmole) in THF and MeOH (2:1) was added LiOH.H<sub>2</sub>O (0.26 mmole) in H<sub>2</sub>O. The mixture was refluxed for 7 hrs and stirred at room temperature for 16 hrs. It was evaporated to dryness. The residue was taken up in water and acidified with 0.1 N HCl. It was extracted with ethyl acetate. The extract was washed with saturated sodium chloride solution, dried over sodium sulfate and evaporated. The crude was passed through a bond-elute (elutents: CH<sub>2</sub>Cl<sub>2</sub> and 5 % acetone in CH<sub>2</sub>Cl<sub>2</sub>) yielding pure 6-(benzyl-*tert*-butoxycarbonyl-amino)-hexanoic acid (12 mg; 57%).

HNMR ( $\delta$ , CDCl<sub>3</sub>): 7.22-7.33 (5H, m), 4.40-4.43 (2H, broad signal), 3.12-3.20 (2H, broad signal), 1.43-1.48 (13H, two broad signals), 1.21-1.25 (2H, m), 1.16 (6H, s).

**EXAMPLE 54**

6-(Benzyl-*tert*-butoxycarbonyl-amino)-2,2-dimethyl-hexanoic acid 4-[4-(dimethylamino-methyleneamino)-2-oxo-2H-pyrimidin-1-yl]-[1,3]dioxolan-2-ylmethyl ester

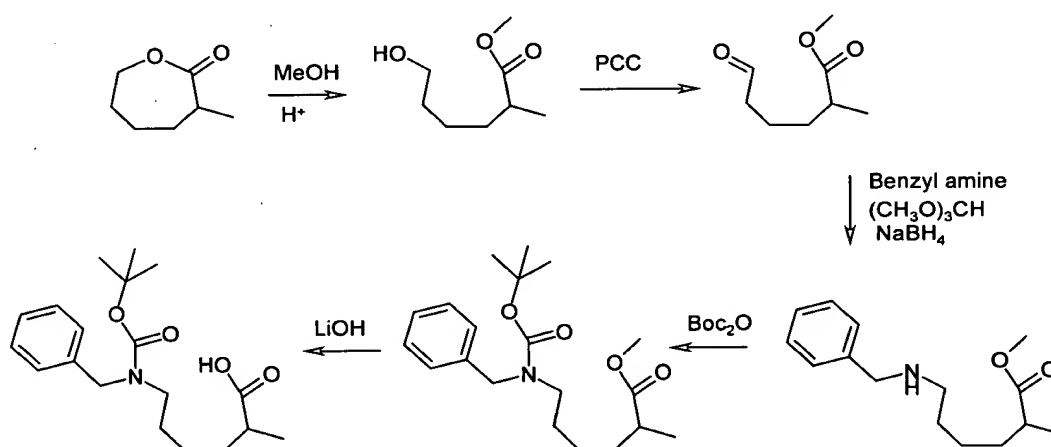
**Procedure:**

5 To a mixture of N'-[1-(2-hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-N,N-dimethyl-formamidinium (0.03 mmole), 6-(benzyl-tert-butoxycarbonyl-amino)-2,2-dimethyl-hexanoic acid (0.03 mmole) and DMAP (0.3 mg) in dichloromethane (0.3 ml) at 0 °C was added EDCI  
 10 (0.063 mmole) in dichloromethane dropwise. It was stirred for 30 minutes at this temperature and at room temperature for 18 hrs. The mixture was diluted with dichloromethane, washed with water and saturated sodium chloride solution. The solution was dried over sodium sulfate and evaporated.  
 15 The crude product was passed through a bond-elute (elutents: dichloromethane, 1 and 2% MeOH in dichloromethane) yielding the ester (28 % yield)

HNMR( $\delta$ , CD<sub>3</sub>OD) : 8.69 (1H, s), 7.96 (1H, d, J = 7.3 Hz),  
 20 7.19-7.32 (5H, m), 6.19-6.23 (2H, m), 5.23 (1H, t, J = 3.2 Hz), 4.49 (1H, dd, J = 3.4, 12.5 Hz), 4.39 (2H, s), 4.22-4.28 (3H, m), 3.22, 3.14 (3H each, s), 1.29-1.47 (15 H, three broad signals), 1.17, 1.16 (3H each, s).

# 25 EXAMPLE 55

**6-(Benzyl-tert-butoxycarbonyl-amino)-2-methyl-hexanoic acid**



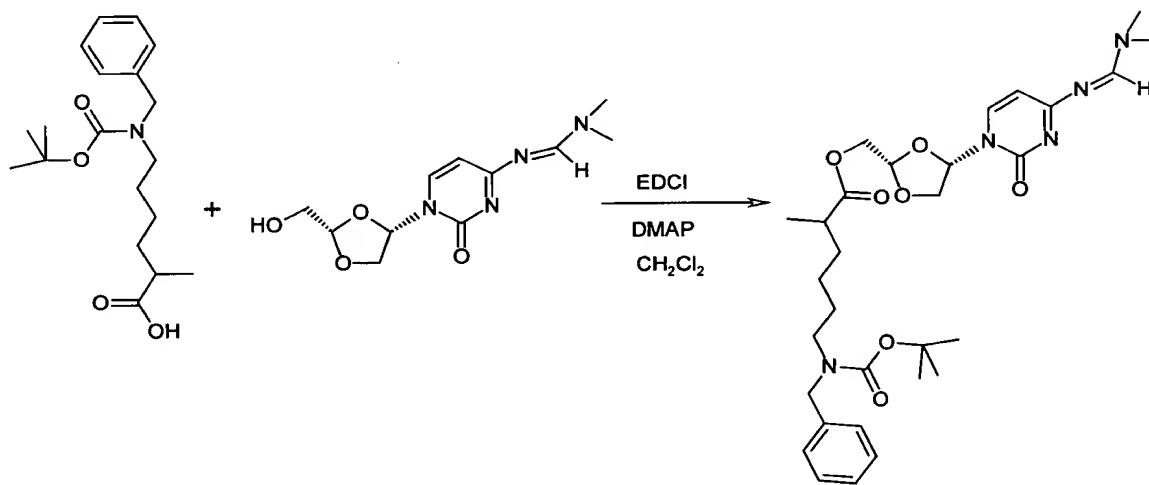


5 **Procedure:**

The procedure to obtain this compound is similar to procedures described in previous examples.

10 **EXAMPLE 56**

**6- (Benzyl-*tert*-butoxycarbonyl-amino) -2-methyl-hexanoic acid 4- [4- (dimethylamino-methyleneamino) -2-oxo-2*H*-pyrimidin-1-yl] - [1,3]dioxolan-2-ylmethyl ester**



(149)

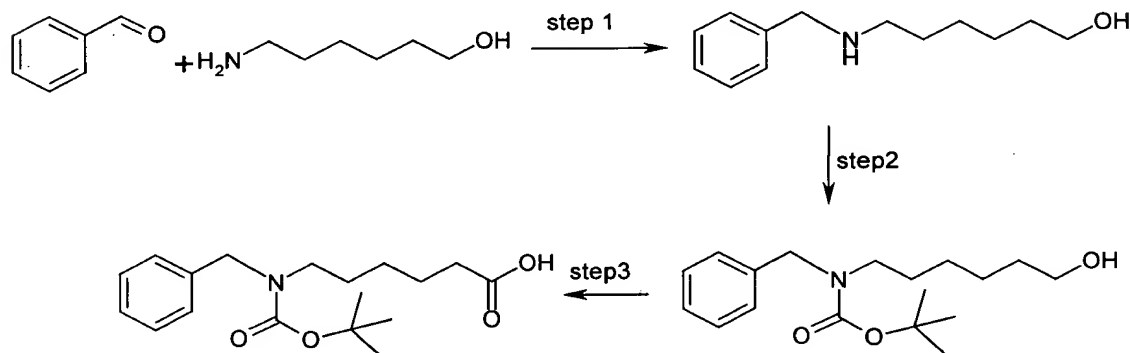
**Procedure:**

To a solution of N'-[1-(2-hydroxymethyl-[1,3]dioxolan-4-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-N,N-dimethyl-formamidinium (0.036 mmole), 6-(benzyl-*tert*-butoxycarbonyl-amino)-2-methyl-hexanoic acid (0.036 mmole) and DMAP (0.4 mg) in dichloromethane at 0 °C was added EDCI (0.078 mmole) in dichloromethane dropwise. The mixture was stirred at 0 °C for 30 minutes and then at room temperature for 2.5 hrs. It was diluted with dichloromethane (50 ml), washed with water and saturated sodium chloride solution. The solution was dried over sodium sulfate and evaporated. The crude was passed through a bond-elute (eluent : CH<sub>2</sub>Cl<sub>2</sub>, 1 and 2 % MeOH in CH<sub>2</sub>Cl<sub>2</sub>) and the pure ester was obtained in 62% yield.

HNMR ( $\delta$ , CD<sub>3</sub>OD): 8.68 (1H, s), 8.02 (1H, two doublets,  $J$  = 7.3 Hz), 7.20-7.32 (5H, multiplets), 6.17-6.25 (2H, m), 5.23-5.25 (1H, broad signal), 4.52 (1H, two dd,  $J$  = 2.4, 12.1 Hz), 4.39- 4.40 (total 2H, broad signals), 4.20-4.31 (3H, m), 3.21, 3.12 (3H each, s), 2.46 (1H, q,  $J$  = 7.0 Hz), 1.20-1.67 (15H, multiplets), 1.12, 1.11 (total 3H, two doublets,  $J$  = 7.0 Hz).

#### EXAMPLE 57

6- (Benzyl-*tert*-butoxycarbonyl-amino)-hexanoic acid



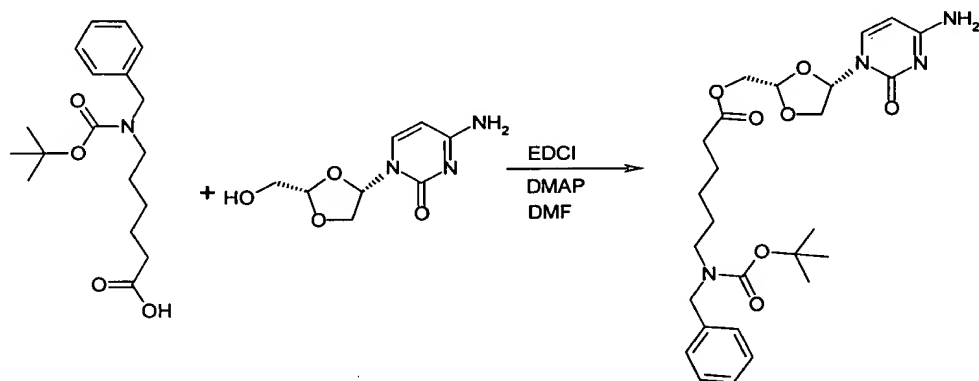
#### Procedure

Steps 1 and 2 were carried out as described in N. Mourier, M. Camplo, G. S. Della Bruna, F. Pellacini, D. Ungheri, J.-C. Chermann and J.-L. Kraus, Nucleosides, Nucleotides & Nucleic Acids, **19** (7), 1057-91 (2000), step 3 was substituted by a Jones oxidation as described in R. N. Rej, J. N. Glushka, W. Chew and A. S. Perlin, Carbohydrate Research, **189** (1989), 135-148.

#### EXAMPLE 58

6- (Benzyl-*tert*-butoxycarbonyl-amino)-hexanoic acid 4-(4-amino-2-oxo-2*H*-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethyl ester

5

**Procedure:**

10 A mixture of 4-amino-1-(2-hydroxymethyl-[1,3]dioxolan-4-yl)-1H-pyrimidin-2-one (0.11 mmole), 6-(benzyl-tert-butoxycarbonyl-amino)-hexanoic acid (0.11 mmole), EDCI (0.156 mmole) and DMAP (3 mg) in DMF was stirred at room temperature for 16 hrs. DMF was removed in vacuum. The residue was taken up in ethyl acetate, washed with water and saturated sodium chloride solution. The solution was dried over sodium sulphate and evaporated. The pure ester was obtained by chromatography over bond-elute (eluent: CH<sub>2</sub>Cl<sub>2</sub>, 2 and 4% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) (17 mg, 31% yield).

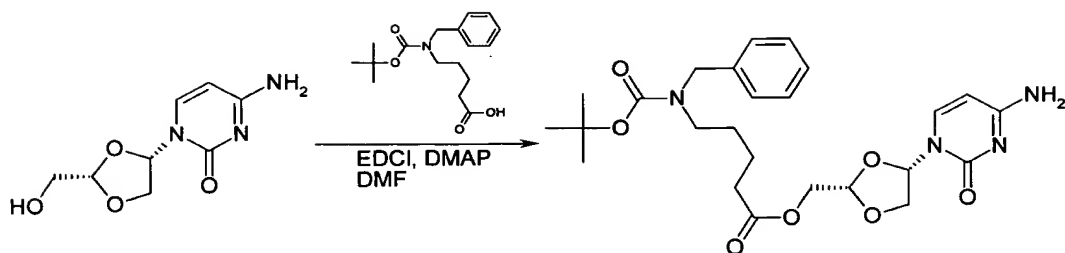
20

HNMR ( $\delta$ , CDCl<sub>3</sub>): 7.78 (1H, broad signal), 7.23-7.34 (5 H, m), 6.28-6.29 (2H, broad signal), 5.70-5.87 (1H, broad signal), 5.21 (1H, broad signal), 4.21-4.48 (6H, two multiplets), 3.20 (2H, broad signal), 2.35 (2H, t,  $J$  = 7.7 Hz), 1.45-1.65 (13H, m), 1.26-1.38 (2H, m).

25

**EXAMPLE 59**

30 **5-(Benzyl-tert-butoxycarbonyl-amino)-pentanoic acid 4-(4-amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]dioxolan-2-ylmethyl ester**



5

(111)

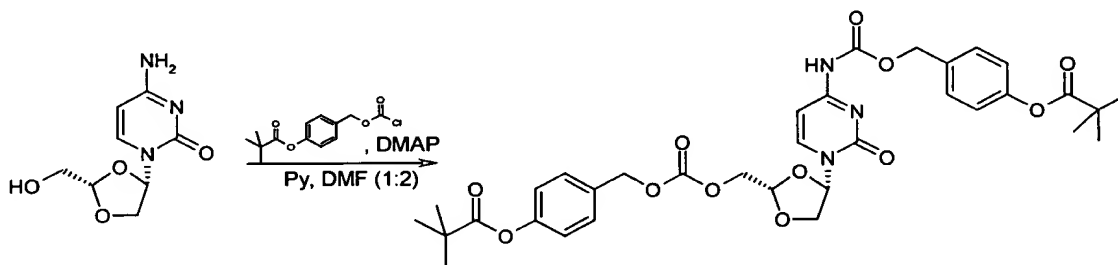
**Procedure:**

4-Amino-1-(2-hydroxymethyl- [1,3]dioxolan-4-yl) -1H-pyrimidin-2-one (0.06 mmol) was treated 5-(Benzyl-tert-butoxycarbonyl-amino)-pentanoic acid (0.07 mmol) (Nucleosides, nucleotides & nucleic acids, 2000, 19 (7), 1057-1091), EDCI (0.09 mmol) and DMAP (catalytic amount) in DMF for 14 hours. The solution was neutralized with NaHCO<sub>3</sub> sat. and extracted with AcOEt. The combined organics layers was dried over sodium sulfate, filtered and concentrated in vacuo. The residue was purified by bond elute (2% MeOH/CH<sub>2</sub>Cl<sub>2</sub> to 10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to afford 36% of 5-(Benzyl-tert-butoxycarbonyl-amino)-pentanoic acid 4-(4-amino-2-oxo-2H-pyrimidin-1-yl) - [1,3]dioxolan-2-ylmethyl ester.

<sup>1</sup>H NMR (CDCl<sub>3</sub>) 7.86 (d, J= 6.4 Hz, 1H), 7.34-7.19 (m, 5H), 6.28 (broad s, 2H), 6.00 (d, J= 6.9 Hz, 1H), 5.07 (s, 2H), 4.50-4.31 (m, 3H), 4.28-4.15 (m, 3H), 3.18-3.08 (m, 2H), 2.17-2.16 (m, 2H), 1.60-1.40 (m, 13H).

**EXAMPLE 60**

2,2-Dimethylpropionic acid 4-(1-{2-[4-(2,2-dimethylpropionyloxy)benzyloxy carbonyloxymethyl]-[1,3]dioxolan-4-yl}-2-oxo-1,2-dihydropyrimidin-4-yl)carbamoyloxymethyl)-phenyl ester (212)



5

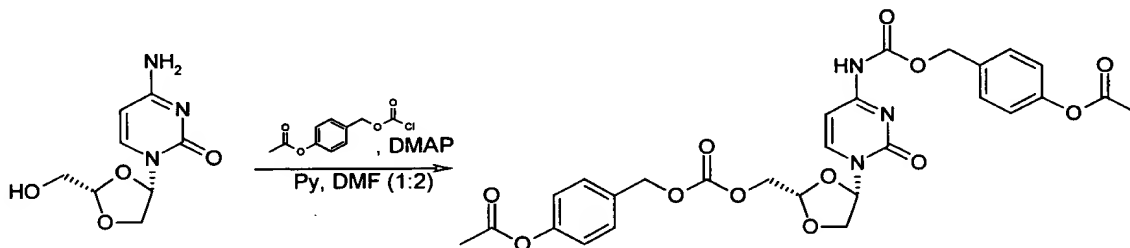
# Procedure:

2,2-Dimethylpropionylchloroformate (1.56 mmol) was added dropwise to a 0°C solution of BCH-4556 (1.30 mmol) and DMAP (1.56 mmol) in dimethylformamide and pyridine and stirred at room temperature for 18h. The reaction mixture was concentrated in vacuo. The oil obtained was partitioned between  $\text{NH}_4\text{Cl}_{\text{sat}}$ /water and dichloromethane. Aqueous layer was extracted with DCM. Organic layers were combined, dried over  $\text{MgSO}_4$ , filtered and concentrated to a yellow gum. The crude residue was purified by silica gel biotage (40S) (40 % EtOAc: 60% hexanes to 80 % EtOAc: 20 % hexanes) to give 1 % yield of 2,2-Dimethylpropionic acid 4-(1-{2-[4-(2,2-dimethylpropionyloxy)benzyloxycarbonyloxymethyl]-[1,3]dioxolan-4-yl}-2-oxo-1,2-dihydropyrimidin-4-ylcarbamoyloxymethyl)-phenyl ester (212) as a white powder.

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ),  $\delta$  ppm: 8.16 (d, 1H,  $J = 7.5\text{Hz}$ ), 7.42-7.38 (m, 4H), 7.23 (d, 1H,  $J = 7.5\text{Hz}$ ), 7.09-7.06 (m, 4H), 6.22-6.21 (m, 1H), 5.24-5.22 (m, 1H), 5.21 (s, 2H), 5.18 (s, 2H), 4.60 (dd, 1H,  $J = 2.6, 12.6\text{Hz}$ ), 4.41 (dd, 1H,  $J = 2.4, 12.6\text{Hz}$ ), 4.30-4.21 (m, 2H), 1.36 (s, 9H), 1.34 (s, 9H).

## EXAMPLE 61

Acetic acid 4-(1-{2-[4-(Acetyloxy)benzyloxycarbonyloxymethyl]-[1,3]dioxolan-4-yl} 2-oxo-1,2-dihydropyrimidin-4-ylcarbamoyloxymethyl)-phenyl ester (202)



5

**Procedure:**

Acetyloxybenzylchloroformate (1.14 mmole, 1,2 eq.) was added dropwise to a 0°C solution of BCH-4556 (0,952 mmole, 1 eq.) and DMAP (1,14 mmole, 1,2 eq.) in dimethylformamide and pyridine and stirred at room temperature for 18h. The reaction mixture was concentrated in vacuo. The oil obtained was partitioned between saturated NH<sub>4</sub>Cl and dichloromethane. Aqueous layer was extracted with dichloromethane. Organic layers were combined, dried over MgSO<sub>4</sub>, filtered and concentrated to a yellow gum. The crude residue was purified by silica gel biotage (40S) (50% EtOAc: 50% hexanes to 100% EtOAc) to give 20,2 mg (4% yield) of the desired product.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>), δ ppm: 8,14 (dd 1H, J = 7,5 and 5,2 Hz), 7,64 (s 1H), 7,40 (m 4H), 7,24 (m 1H), 7,10 (m 4H), 6,20 (t 1H, J = 5,0 Hz), 5,19 (m 5H), 4,58 (m 2H), 2,30 (s 3H), 2,28 (s 3H).

### Example 62 - Cell Proliferation Assays/ NT Inhibitor Studies

The chemosensitivity of suspension cells lines (e.g., CEM or CEM-derivatives) is assessed using the CellTiter 96<sup>®</sup> proliferation assay. Cells are seeded in 96-well plates (8 replicates) in three separate experiments and exposed to graded concentrations (e.g., 0.001-100 μM) of a nucleoside of interest (e.g., cytarabine, gemcitabine or troxacitabine), for 48 h. Chemosensitivity is expressed as 50% (EC<sub>50</sub>) of the dose response curve determined, e.g., using GraphPad Prism 2.01 (GraphPad Software, San Diego,

5 CA). Adherent cell lines (e.g., DU145 or DU145<sup>R</sup>) are seeded (~10<sup>5</sup> cells) in triplicate dishes, 24 h before drug exposure. Growth inhibition is determined by trypsinization and counting cells electronically.

10 In this example, troxacitabine is shown to enter cells by a mechanism other than via the NT, *es* (defective in CEM/ARA89C), or via the four other NTs which are not present in CEM cells, *ei*, *cit*, *cif*, and *cib* (See, e.g., Ullman (1989). *Advances in Experimental Medicine & Biology* 253B: 415-20). This is consistent with entry into the  
15 cells by passive diffusion. The ability of troxacitabine to inhibit cell proliferation of CEM and CEM-derivative cell lines was directly compared to other cytosine-containing nucleoside analogs, gemcitabine and cytarabine, in a cell proliferation assay (See Table 1). The growth of  
20 CEM cells was inhibited by all three nucleoside analogs, and troxacitabine was 16 and 8-fold less toxic than cytarabine and gemcitabine, respectively. The presence of the *es* transport inhibitor, NBMPR, significantly increased resistance of CEM cells to gemcitabine and cytarabine but  
25 not to troxacitabine. CEM cells are reported to exhibit primarily *es*. Therefore, this example suggests that that the uptake of troxacitabine is less dependent on the presence of a functional hENT1 transporter (*es*) in CEM cells than cytarabine or gemcitabine. In addition, there  
30 was a much lower level of resistance observed for the nucleoside-transport deficient CEM/ARAC8C cells exposed to troxacitabine (8-fold) compared to cytarabine (1150-fold) or gemcitabine (431-fold), further implying lack of transport of troxacitabine (by *es* NT). Taken together, the  
35 data suggested that troxacitabine has a different uptake mechanism than cytarabine and gemcitabine. This again is consistent with entry into the cells by passive diffusion.

5 Table 1. Comparative chemosensitivities of CEM and CEM-  
derivative cell lines to troxacitabine,  
gemcitabine and cytarabine.

10 Cultures were exposed to graded concentrations (0.001-  
100  $\mu$ M) of cytarabine, gemcitabine or troxacitabine  
for 48 h. Chemosensitivity was measured using the  
Promega CellTiter 96 cell proliferation assay and  
expressed as 50% of the dose response curve ( $EC_{50}$ ).  
15 The effect of the es transport inhibitor, NBMPR (100  
nM) on the  $EC_{50}$  values of CEM cells exposed to  
cytarabine, gemcitabine or troxacitabine was also  
determined. Each value represents the average ( $\pm$   
standard deviation) of three separate experiments  
(each experiment had 8 replicates).

20

Cell line	Cytarabine	Gemcitabine	Troxacitabine
CEM	0.01 0.002	$\pm$ 0.02 .0004	$\pm$ 0.16 $\pm$ 0.012
CEM + NBMPR	0.05 0.006	$\pm$ 0.07 0.018	$\pm$ 0.21 $\pm$ 0.019
CEM/ARAC8C	11.50 2.654	$\pm$ 8.63 0.881	$\pm$ 1.18 $\pm$ 0.315
CEM/dCK	>50	>50	>100

#### EXAMPLE 63 - Cellular Uptake Assays.

25 Measurements of nucleoside uptake are performed by  
conventional methods, as described, e.g., in Rabbani et  
al. (1998) *Cancer Res.* 58: 3461; Weitman et al. (2000).  
*Clinical Cancer Res.*, 6:1574-1578; or Grove et al. (1996).  
*Cancer Res.*, 56: 4187-4191. Briefly, for adherent cells,  
30 uptake assays are conducted at room temperature under  
zero-trans conditions in either sodium-containing  
transport buffer (20 mM Tris/HCl, 3 mM  $K_2HPO_4$ , 1 mM  
MgCl<sub>2</sub>.6H<sub>2</sub>O, 2 mM CaCl<sub>2</sub>, 5 mM glucose and 130 mM NaCl, pH  
7.4, 300  $\pm$  15 mOsm) or sodium-free transport buffer with  
35 NaCl replaced by N-methyl-D-glucamine. Cells are washed  
twice with the appropriate transport buffer and then



5 either processed immediately, or in some experiments,  
 incubated with transport inhibitors, NBMPR (100 mM),  
 dipyridamole (20  $\mu$ M) or dilazep (100  $\mu$ M) during the second  
 wash at room temperature for 15 min before the uptake  
 assay. Precisely timed intervals are initiated by adding  
 10 transport buffer containing [ $^3$ H]troxacitabine or  
 [ $^3$ H]uridine and terminated by immersion in ice-cold  
 transport buffer. After the plates are drained, the cells  
 are lysed with 5% Triton X-100 and mixed with Ecolite  
 scintillation fluid to measure the cell-associated  
 15 radioactivity (Beckman LS 6500 scintillation counter;  
 Beckman-Coulter Canada, Mississauga, ON). Uptake at the  
 zero time-point is determined by treating cells for 10 min  
 at 4°C with transport buffer containing 100  $\mu$ M dilazep,  
 then adding the radioactive nucleoside for 2 s before  
 20 reaction termination as described above. Uptake assays  
 for suspension cells are conducted in microfuge tubes and  
 permeant fluxes are terminated using the ``inhibitor-oil~~✕~~  
 stop method; dilazep is used at a final concentration of  
 200  $\mu$ M. Uptake at the zero time-point is determined by  
 25 adding cells to cold transport buffer containing  
 radiolabeled permeant and dilazep, and immediate  
 centrifugation. Cell pellets are lysed and cell-  
 associated radioactivity measured.

30 EXAMPLE 64 - NT Inhibitor Studies/ Competition with an  
 excess of the nucleoside of interest, itself, in non-  
 radioactive form

CEM cells: CEM cells contain primarily one type of  
 35 nucleoside transport activity (es), and the functionality  
 of this transporter (hENT1) was first demonstrated by the  
 uptake of the physiological substrate, uridine (Fig.1A),  
 using methods as described in Example 29. The transport of  
 [ $^3$ H]uridine was inhibited in the presence either of the  
 40 hENT1 inhibitor, NBMPR, or excess non-radioactive uridine.  
 [ $^3$ H]troxacitabine was taken up to a lesser degree over the  
 6-min time course in CEM and in CEM/ARAC8C cells (Fig. 1B).

5 Lack of [<sup>3</sup>H]uridine uptake in the latter cell line demonstrated the absence of functional hENT1 transporters. The data suggest that troxacitabine uptake in CEM cells is not mediated by *es* activity and is consistent with it being taken up by passive diffusion.

10

DU145 cells: The presence of functional *es*-mediated transport (hENT1) in DU145 cells was first demonstrated in a cellular uptake assay with 10  $\mu$ M [<sup>3</sup>H]uridine, as a control substrate in the presence and absence of the hENT1 inhibitor, NBMPR. In the presence of NBMPR, total [<sup>3</sup>H]uridine uptake over a 6-min time course was inhibited by ~75% (Fig. 2A). In contrast, low levels of [<sup>3</sup>H]troxacitabine were taken up and uptake was not affected by the presence of NBMPR (Fig. 2B). The results are consistent with the uptake of troxacitabine observed in CEM cells and provide further evidence that troxacitabine is a very poor substrate for hENT1, and probably enters the cell by passive diffusion.

25 HeLa cells: [<sup>3</sup>H]Troxacitabine and [<sup>3</sup>H]uridine cellular uptake by hENT2 (*ei* NT) in HeLa cells. In the presence of the hENT1 inhibitor, NBMPR, the functionality of hENT2 was first demonstrated in a cellular uptake assay with 10  $\mu$ M [<sup>3</sup>H]uridine (Fig.3A). A high total uptake of uridine was observed over a long time course of 240 min of about 1200 pmol/10<sup>6</sup> cells. In an expanded scale over the same time period, low levels of [<sup>3</sup>H]troxacitabine were taken up with a total uptake of about 10 pmol/10<sup>6</sup> cells, 120-fold lower than uridine (Fig 3B). In the presence of nucleoside transport inhibitors, NBMPR, dilazep, and dipyridamole or excess non-radioactive troxacitabine, no substantial inhibition of troxacitabine uptake was observed. Taken together, the results demonstrate that compared to uridine, troxacitabine is a very poor substrate for hENT2. Furthermore, the fact that an excess of unlabeled troxacitabine failed to inhibit the uptake of the labeled troxacitabine indicates that

5 troxacitabine is not mediated by a nucleoside transporter, i.e., that it enters the cells by passive diffusion.

DU145 cells: This experiment is designed to show whether [3H]L-troxacitabine (10µM) is taken up by DU145 cells and if  
10 the rate of uptake is affected by the addition of high concentrations (1 mM) of non-radioactive troxacitabine. The results show that the uptake of [3H]L-troxacitabine is very slow during both short (0-30s) and prolonged exposures (0-4 h). The addition of non-radioactive troxacitabine has  
15 no significant effect on the uptake of [3H]L-troxacitabine, an indication that uptake in these cells is not mediated by a NT, but instead is taken up by passive diffusion.

EXAMPLE 65 - Uptake by hCNT1, hCNT2 and hCNT3

20 [3H]Troxacitabine and [3H]uridine uptake by recombinant hCNT1 and hCNT2 in transient-transfection assays in HeLa cells:

Expression plasmids encoding recombinant hCNT1 and hCNT2  
25 are prepared using conventional methods. Genes encoding the hCNT1 and hCNT2 transporter proteins are subcloned from the plasmids pMHK2 (Ritzel et al. (1997). *Am. J. Physiology* 272: C707-C714) and pMH15 (Ritzel et al. (1998). *Mol Membr Biol.* 15: 203-11) into the mammalian expression vector,  
30 pcDNA3, to produce pcDNA3-hCNT1 (Graham et al. (2000). *Nucleosides Nucleotides Nucleic Acids* 19: 415-434) and pcDNA3-hCNT2. The expression vectors are separately introduced into actively proliferating HeLa cells, following conventional methods. See, e.g., Fang et al  
35 (1996). *Biochemical Journal* 317: 457-65.

Recombinant hCNT1 and hCNT2 were separately introduced into HeLa cells by transient transfection of pcDNA3 plasmids containing the coding sequences of the relevant nucleoside  
40 transporter protein. After transfection, functionality of

5 each transporter was demonstrated by comparing the uptake  
of 10  $\mu\text{M}$  [ $^3\text{H}$ ]uridine in the presence of the equilibrative  
transporter (hENT1, hENT2) inhibitor, 100  $\mu\text{M}$  dilazep, to  
cells transfected with the empty vector pcDNA3 control  
plasmid (Fig. 4). Uptake of 10  $\mu\text{M}$  [ $^3\text{H}$ ]troxacitabine was not  
10 mediated either by hCNT1 or by hCNT2.  
Troxacitabine uptake by *cib*-activity (hCNT3) in  
differentiated HL-60 cells:

15 The ability of a high concentration (100-fold) of non-  
radioactive troxacitabine to inhibit the uptake of  
[ $^3\text{H}$ ]uridine by hCNT3 was examined in a differentiated HL-60  
model system [Ritzel et al. (2000), *supra*]. Under these  
conditions, troxacitabine had no effect on uridine uptake  
and suggested that troxacitabine was not substrate of  
20 hCNT3.

The examination of troxacitabine uptake in several cell  
lines has shown that uptake is not mediated by any of the  
characterized equilibrative (hENT1, hENT2) or sodium-  
25 dependent (hCNT1, hCNT2, hCNT3) nucleoside transporters.  
The low uptake observed for troxacitabine is consistent  
with a diffusion model.

30

5

Table of IC50 Values ( $\mu\text{M}$ ) for Controls  
 Exposition of 24hr to drug, wash, incubated for another 48hr (total of 72hr  
 assay)  
 (3H-Thymidine Incorporation Assay)

10

IC50 in  $\mu\text{M}$  (3H-TdR incorporation at  
 72hr)

Compound	H-460 24h	MCF-7 24h	SF-268 24h	CCRF-CEM 24h	CEM/dCK- 24h	Factor*
Gemcitabine	0.0084	0.0090	0.0030	0.0035	51	14 571
	0.0140	0.0048	0.0110	0.0064	51	7 969
	0.0420	ND	0.0094	0.0034	30	8 824
	0.0083	0.0019	0.0077	0.0086	41	4 767
	0.0066	0.0083	0.0073	0.0092	30	3 260
	0.0100	0.0024	0.0110	0.0048	77	16 041
	0.0110	0.0049	0.0100	0.0094	85	9 043
	0.0160	0.0093	0.0130	0.0100	86	8 600
	0.0094	0.0100	0.0140	0.0086	80	9 302
	0.0097	0.0086	0.0100	0.0092	>100	10 870
	0.0110	0.0056	0.0091	0.0100	91	9 100
	0.0110	0.0060	0.0094	0.0092	93	10 109
	0.0110	0.0087	0.0090	0.0084	92	10 952
	0.0130	0.0120	0.0081	0.0120	>100	>8 333
	0.0041	0.0087	0.0045	0.0028	41	14 643
	0.0079	0.0059	0.0075	0.0079	87	11 013
	0.0055	0.0031	0.0045	0.0200	61	3 050
	0.0110	0.0100	0.0083	ND	88	ND
	0.0100	0.0094	0.0100	0.0061	66	10 820
	0.0091	0.0029	0.0037	0.0051	34	6 667
	0.0074	0.0051	0.0089	0.0090	40	4 444
	0.0091	0.0068	0.0078	0.0096	48	5 000
	0.0100	0.0089	0.0086	0.0100	72	7 200
	0.0110	0.0034	0.0100	0.0099	36	3 636
	0.0083	0.0041	0.0029	0.0073	>100	>13700
Average	0.011 $\pm$ 0.007	0.0068 $\pm$ 0.002 8	0.0086 $\pm$ 0.0027	0.0084 $\pm$ 0.0035	66 $\pm$ 24	8618 $\pm$ 3614

Cytosine Arabinoside	0.0140	0.0088	0.140	0.0024	21	8 750
	0.0190	0.0220	0.450	0.0034	24	7 059
	0.0500	ND	0.470	0.0030	23	7 667
	0.0100	0.0098	0.077	0.0028	18	6 428
	0.0130	0.0100	0.320	0.0037	19	5 135
	0.0130	0.0140	0.033	0.0032	29	8 906
	0.0160	0.0160	0.300	0.0049	27	5 510
	0.0360	0.0170	0.300	0.0068	32	4 706
	0.0078	0.0200	ND	0.0280	>100	6 250
	0.0990	0.1000	2,100	0.0370	>100	2 700
	0.1500	0.1500	1,900	0.0350	>100	2 857
	0.1200	0.1700	0.890	0.0410	>100	2 439
	0.0990	0.1000	3,600	0.0250	>100	4 000
	0.1400	0.1500	1,200	0.0470	>100	>2 128
	0.0350	0.0960	0.120	0.0089	>100	>11 236
	0.0160	0.1100	1,600	0.0590	>100	1 695
	0.0540	0.0340	0.930	0.0084	>100	>11 905
	0.1100	0.1000	2,600	ND	>100	ND
	0.0750	0.0810	1,100	0.0100	41	4 100
	0.0160	0.0095	0.770	0.0056	41	7 321
	0.0200	0.0210	0.660	0.0094	40	4 255
	0.0160	0.0270	0.920	0.0092	78	8 478
	0.0780	0.0520	0.720	0.0100	59	5 900
	0.0370	0.0120	0.490	0.0071	40	5 634
	0.0250	0.0310	0.110	0.0053	75	14150
Average	0,052±0,045	0,061±0,052	0,94±0,89	0,016±0,017	62±35	5872±2783
BCH-4556	0,040 (72h)	0,066 (72h)	0,096 (72h)	0,076 (24h)	>100 (24h)	>1315
	0.130	0.005	0.27	0.045	56	1 244
	0.140	0.140	0.33	0.040	>100	2 500
	0.049	ND	0.43	0.091	>100	1 099
	0.110	0.140	0.17	0.073	>100	1 370
	0.086	0.180	0.24	0.065	>100	1 538
	0.150	0.190	0.68	0.120	>100	833
	0.110	0.200	0.33	0.099	>100	1 010
	0.170	0.160	0.41	0.080	>100	1 250
	0.100	0.420	ND	0.028	>100	3 571
	0.140	0.160	0.40	0.100	>100	1 000
	0.180	0.340	0.74	0.096	>100	1 041
	0.140	0.015	0.15	0.100	>100	1 000
	0.110	0.310	0.71	0.083	>100	1 200
	0.160	0.280	0.49	0.130	>100	>769
	0.100	0.150	0.19	0.013	>100	>7 692
	0.140	0.210	0.63	0.063	>100	>1 587
	0.078	0.097	0.51	0.021	>100	>4 762
	0.150	0.220	0.66	ND	>100	ND
	0.160	0.140	0.59	0.072	>100	>1 389
	0.110	0.150	0.47	0.086	>100	>1 163
	0.130	0.220	0.66	0.059	>100	>1 695
	0.110	0.170	0.38	0.100	>100	>1 000
	0.130	0.220	0.53	0.074	>100	>1 351
	0.100	0.043	0.36	0.087	>100	>1 150
	0.180	0.031	0.11	0.0053	>100	>1 136
	0,12±0,03	0,18±0,10	0,44±0,18	0,078±0,028	>100	1792±1584
27	0,0053 (72h)	0,0073 (72h)	0,023 (72h)	nd	nd	nd

275	0,0012 (72h)	0,0044 (72h)	0,013 (72h)	0.0056	51.6	9,214
276	0.025 (72h)	0.0017 (72h)	0,018 (72h)	0.028	26.8	957
277	0.20 0.29	0.013 0.016	0.21 0.19	0.049 0.100	>100 >100	2 040 >1 000
278	0.0024 (72h) 0,079	0.023 (72h) 0,038	0,013 (72h) 0,093	0,028 0,028	71,2 91	2543 3250
279	0,073 (72h) 0,58	0,021 (72h) 0,24	0,044 (72h) 0,39	0,026 0,083	48,2 >100	1854 >1205
280	1.9	3.1	18	1.9	>100	>53
38	0.34	1	0.90	0.11	>100	909
39	0.16 0.12	0.38 0.12	0.32 0.39	0.047 0.062	>100 >100	2 128 1 667

40	0.32	0.070	0.90	0.089	>100	1,123
41	40	91	>100	21	>100	5
42	0.010 0.007	0.014 0.005	0.022 0.026	0.0022 0.0023	82 >100	37 272 43 378
43	0.010	0.0041	0.029	<0,0001	>100	1,000,000
44	0.37	0.97	0.89	0.077	>100	1,300
45	3.2	2.7	9	1.6	>100	63
46	0.086	0.16	0.56	0.060	>100	1,667
47	1.8	2.4	38	2.9	>100	34



48	0,34 0,59	1,2 4,7	0,56 23	0,17 3,5	>100 >100	588 >29
49	4.5	8.8	7.1	0.57	>100	175
50	1.2	0.82	1.3	0.17	>100	588
51	0.83	0.57	0.86	0.024	47	1,958
52	0.0068	0.088	0.032	0.0012	0.48	400
53	8.9	10	10	2	37	19
54	0.17	0.50	0.70	0.12	65	542
55	0.029	0.0078	0.047	0.012	64	5,333

56	7	2	25	1.6	>100	63
57	0.0061	0.019	0.047	0.0048	32	6,667
58	0.012	0.016	0.13	0.014	38	2,714
59	1.4	0.19	0.69	0.54	>100	185
60	2,0 3,1	0,86 0,95	0,86 4,7	0,29 0,31	2,9 1,8	10 6
61	0.13 0.20 0.076	0.0770 0.0088 0.015	0.054 0.013 0.064	0.040 0.013 0.0074	>100 >100 >100	> 2 500 > 7 692 >13 513
62	0.89	1.7	4.3	0.35	>100	288
63	0.11	0.37	0.076	0.036	>100	2,778

64	0.0017	0.0044	0.0071	0.0018	3.6	2,000
65	0.011	0.012	0.033	0.0039	26	6,667
66	<0,00010 0.00025	<0,0001 0.000074	<0,0001 0.0011	<0,00010 0.000009	3 >0.1	>28 000 11 627
67	0.082	ND	0.40	0.18	>100	556
68	0.019	0.076	0.21	0.030	>100	3,333
69	0.045	0.028	0.050	0.0069	43	6,231
70	0.036	0.047	0.27	0.0088	30	3,409
71	0.31	0.13	0.81	0.18	>100	556

72	0.018 0.027	0.015 0.017	0.130 0.075	0.0160 0.0062	23 23	1 450 3 710
73	0.27	0.26	0.030	0.10	99	990
74	5.2	1.4	4.4	0.33	1.3	4
75	>100	64.00	>100	>100	>100	1
76	>100	>100	>100	>100	>100	1
77	0.059	0.030	0.38	0.054	74	1,370
78	0.042	0.045	0.095	0.037	13	351
79	0.12	0.17	0.16	0.014	63	4,500

80	1.8	0.67	3.5	0.46	>100	217
81	3.1	2.2	7.9	1.2	>100	83
82	0.17	0.12	0.30	0.053	>100	1,887
83	0.054	0.083	0.26	0.022	>100	4,545
84	0.014	0.0094	0.36	0.012	60	5,000
85	0.69	6.8	16	2.6	>100	38
86	0.0020	0.0019	0.013	0.0011	4	3,636
87	0,41 1,2 0,48	0,6 1,9 1,2	0,65 5,2 1,9	0,10 0,42 0,39	>100 >100 >100	>1 000 >238 >256

88	0.14	0.19	0.61	0.088	82	931
89	3.8	0.22	11	2.5	>100	40
90	95	61	>100	65	>100	1.5
91	0.63	1.8	5.5	2.8	>100	36
92	2.1	1.6	4.2	1.3	>100	77
93	0.04 74	>100 13.6	>100 >100	19 4.2	>100 >100	>5 >24
94	0.025 14	24 13	38 92	17 6	51 85	3 16
95	<0.0001 nd	0.15 0.10	0.61 0.25	0.240 0.057	30 86	123 1 503

96	0.0061 1.5	0.19 0.21	1.4 9.6	1.8 1.9	>100 >100	>56 >52
97	N.D 22	5,0 4,0	56 25	9.2 5.9	>100 >100	>11 >19
98	nd 36 11	0.13 0.15 0.22	>100 2.2 2.3	35 22 61	>100 >100 >100	>3 >4 >3
99	N.D.	6.3	33.0	5	>100	>20
100	nd 0.030 0,044 nd	2.70 1.40 0,96 0,25	4.80 0.09 5,80 1,00	2.70 0.52 2,50 0,64	19 55 45 15	7 105 18 23
101	0.33	0.41	2.1	0.36	16	44
102	0.19	1.7	1.0	0.41	11	27
103	0.052	0.018	0.063	0.011	50	4,545

104	0.27	0.47	0.47	0.21	>100	>476
105	0.080	0.068	0.071	0.033	79	2 393
106	0.014	0.037	0.095	0.010	46	4,600
107	0.0280 0.0094 0.0340 0,0200 0,0037 0,0084	0.012 0.019 0.030 0,013 0,023 0,035	0.220 0.078 0.034 0,068 0,071 0,260	0.0120 0.0056 0.0088 0,0200 0,0140 0,0210	37 30 83 82 59 20	3 100 5 428 9 432 4 100 4 214 952
108	1.8	27	3.8	3.4	>100	>29
109	2.6	31	4.8	1.0	>100	>100
110	0.0010	0.010	0.0049	0.0013	4.3	3 307
111	0.00013	0.00026	0.0021	0.00020	2.6	13000

104  
105  
106  
107  
108  
109  
110  
111



112	0.011	0.016	0.0067	0.0058	0.057	10
113	0.24	0.48	1.1	0.060	>100	>1 667
114	0.066	0.017	0.041	0.016	8	500
115	0.38	0.15	0.62	0.20	>100	>500
116	1.4	0.11	2.5	0.38	>100	>263
117	0.46	0.46	0.68	0.18	89	494
118	0.022	0.077	0.16	0.028	>100	>3 571
119	17	27	94	56	96	~2

120	>100	64	>100	>100	>100	1
121	28	37	>100	17	>100	>6
122	1.9	0.21	0.57	0.71	61	86
123	1.0	1.4	2.0	0.87	15	17
124	13	14	49	14	27	~2
125	0.24	0.016	0.60	0.072	7	97
126	0.0041	0.0020	0.0085	0.0016	13	8,125
127	35.0 4,9	16 15	23 >100	15 22	>100 >100	>7 >4,5

128	0.14	0.090	0.17	0.22	>100	>454
129	0.15	0.020	0.20	0.072	15	208
130	0.058	0.050	0.11	0.057	75	1,316
131	0.11	0.10	0.012	0.021	83	3,952
132	0.0021 0.0190 0,0130 0,0016	0.0011 0.0200 0,0130 0,0010	<0.0001 0.0180 0,0130 0,0045	<0.00010 0.00091 0,00370 <0.00010	8 >1 11 10	>80 000 >1 100 2 973 >100 000
133	0.021	0.10	0.016	0.027	31	1,148
134	12	11	3	7	20	3
135	0,15 9,00	0,23 11,0	0,25 ND	0,097 4,1	59 19	608 5

136	9	12	3	4	>100	>25
137	6.00 0,35	17.0 5,1	18,4 16.0	5.0 6,5	84 53	17 8
138	0.92	1.5	2.1	0.53	58	109
139	0.81 0.51	1.4 1.7	1.3 1.7	0.40 0.42	>100 >100	>250 >250
140	10	20	3	11	>100	>9
141	0.034	0.066	0.040	0.019	69	3,632
142	0.038	0.029	0.13	0.0072	46	6,389
143	0.012	0.0037	0.14	0.0039	32.0	8,205

144	3	5.2	1.9	0.71	78	110
145	0.24	0.77	0.12	0.084	69	821
146	0.78	1.2	0.028	0.13	50	385
147	0.060	0.11	0.017	0.025	>100	>4 000
148	36	6.30	9.90	6.3	24	4
149	<0.0001 0.0028	0.00150 0.00039	<0.0001 0.0070	<0.00010 0.00012	2 >1,8	>19 000 >15 000
150	0.96	1.6	1.3	0.13	90	692
151	9.7	8.3	4.4	0.59	>100	>169

152	3.5	3.0	31.00	0.79	>100	>127
153	46	39	59	0.21	>100	>476
154	0.76	1.6	4.4	0.14	>100	>714
155	1,6 0,093 0,43	3,7 0,060 0,76	5,9 0,97 1,7	0,10 0,15 0,54	>100 >100 >100	>1 000 > 667 > 185
156	0.12	0.068	0.93	0.0070	81	11,571
157	0.024	0.55	2.2	0.012	>100	>8 333
158	0.63	0.040	3.7	0.094	58	617
159	0.87	0.72	1.6	0.38	>100	>263

160	0.92	0.36	1.2	0.36	>100	>278
162	8.4 6.4 9,2 2,9	9.4 3.9 5,7 3,6	1.1 7.0 12 17	2.2 2.8 3,3 4,1	>100 >100 >100 >100	>44 >36 >30 >24
163	0.0092	0.033	0.025	0.0033	27	8,182
164	0.13	0.14	0.28	0.060	>100	1 667
165	3.4	10	16	1.8	>100	>56
166	0.0073 0.0044 0,0180 0,0170	0.0012 0.0014 0,0090 0,0110	0.0046 0.0092 0,0580 0,0640	0.0001 0.0077 0,0047 0,0024	10 >1 10 >100	>90 000 >130 2 128 >41 667
167	0,160 0,062 0,230	0,20 0,12 0,30	0,64 0,12 0,54	0,073 0,031 0,110	10 >100 12	137 3 225 109
168	96 25 45	16 2,4 44	98 31 59	31 22 20	>100 >100 >100	>3 >4 >5

169	8.2	5.1	7.1	2.0	>100	>50
170	0.63	0.49	1.0	0.21	>100	>476
171	45	41	82	38	>100	>2.6
172	0,014 0,015	0,019 0,036	0,0037 0,0210	0,0074 0,0085	2 5	270 588
173	6.1	17	2.0	2.6	>100	>38
174	11	21	38	9.0	>100	>11
175	6.3	3.1	32	3.5	>100	>29
176	0,040 0,043	0,094 0,032	0,057 0,032	0,014 0,011	38 68	2 714 6 182



177	0.19	0.22	0.92	0.095	>100	>1 052
178	88	5.8	41	25	>100	>4
179	1.7	2.8	0.56	2.4	>100	>42
180	>100	65	49	>100	>100	>1
181	0.14	0.49	0.17	0.037	>100	>2700
182	0.13	0.22	0.21	0.047	>100	>2100
183	0.037	0.038	0.12	0.018	45	2,500
184	0.94	0.92	1.1	0.81	40	49

185	0.059	0.064	0.054	0.066	17	258
186	<0.0001 <0.0001 0,0039	0,0300 0,0210 0,0062	0,0270 0,0017 0,0770	0,0087 0,0220 0,0049	>100 >100 >100	>11 494 > 4 545 >20 408
187	0,0014 0,0011	0,0042 0,0051	0,0200 0,0080	0,0017 0,0016	4,1 0,66	2 412 413
188	0,097 0,068 0,120	3,0 3,8 4,9	0,46 2,40 2,40	0,79 1,50 1,10	>100 >100 >100	>127 > 67 > 91
189	0,00120 0,00068	0,0033 0,0037	0,0092 0,0016	0,0021 0,0010	2,8 1,3	1333 1 300
190	0,0061 0,0039	0,027 0,016	0,0400 0,0056	0,0084 0,0036	22 9,8	2 619 2 722
191	<1E-04 <1E-11 ND	<1E-04 <1E-11 ND	<1E-04 <1E-11 ND	<1E-04 <1E-11 1,6E-11	0,54 >1E-04 11	>5 400 >1E07 7,0E11
192	0.29	0.0016	0.40	0.0084	48	5,714

193	0.64	0.16	2.0	0.059	>100	>1 695
194	0.011	0.0040	0.041	0.0024	10	4 167
195	1.1	1.9	1.5	0.064	>100	>1 563
196	<1E-04 1.1E-08 ND	<1E-04 <1E-11 ND	<1E-04 2.5E-07 ND	<1E-04 <1E-11 1,2E-06	2,5 >1E-04 26	>25 000 >1E07 2,2E07
197	<1E-04 <1E-11 ND	<1E-04 <1E-11 ND	<1E-04 <1E-11 ND	<1E-04 <1E-11 ND	0,94 >1E-04 11	>9 400 >1E07 ND
198	<1E-04 1.4E-08 ND	<1E-04 1.2E-05 ND	<1E-04 1.0E-07 ND	<1E-04 1.1E-08 ND	2,1 >1E-04 17	>21 000 >10 000 ND
199	0.033	0.21	0.0078	0.0094	>100	>10 638
200	0.30	1.1	0.12	0.31	72	232

201	17	18	7.3	14	>100	>7
202	<1E-04 2,1E-05	<1E-04 ND	<1E-04 1,2E-05	<1E-04 ND	0,1 1,1	>1 000 ND
203	<1E-04 ND	<1E-04 ND	<1E-04 ND	<1E-04 3,3E-04	1,3 8,6	>13 000 26 060
204	0.015	0.0086	0.025	0.012	19	1 600
205	0.28	0.90	0.10	0.26	>100	>385
206	0.012	0.056	0.043	0.0090	80	8,889
207	0.0061	0.0044	0.0023	0.0027	15	5,556
208	<1E-04 0,0027	<1E-04 0,00063	<1E-04 0,0062	<1E-04 0,000052	1,42 11	>14 000 211 538

209	0.31	1.3	0.59	ND	>100	ND
210	0.0026	0.0050	0.26	ND	>100	ND
211	≤0,0001 0,0000086 0,0000400	≤0,0001 0,000015 0,000030	≤0,0001 0,00016 0,00087	ND 0,000027 0,000053	0,71 >1 >0,1	ND >3 704 >1 887
212	0.00011	0.00059	0.018	ND	3.5	ND
213	≤0,0001	0.00027	0.012	ND	1.1	ND
214	9.4	9.4	89	ND	>100	ND
215	3.9	33	96	ND	>100	ND
216	0.00088	≤0,0001	0.018	ND	14	ND

217	≤0,0001	≤0,0001	0.00013	ND	1.2	ND
218	0.0091	0.052	0.081	ND	60	ND
219	≤0,0001	≤0,0001	0.00012	ND	2.1	ND
220	0.0034	0.029	0.042	0.0035	>100	>28 571
221	0.43	0.39	1.6	0.13	>100	>769
222	0.21	0.19	0.85	0.11	>100	>909
223	0.035	0.15	0.25	0.062	>100	>1 613
224	5.3	6.9	21	0.10	>100	>1 000

225	11	11	43	0.88	>100	>113
226	0,00063 0,02600	0,0017 0,0330	0,035 0,016	0,00076 0,02100	28 >0,1	36 842 > 5
227	0.84	0.012	3.0	0.043	22	512
228	0.68	1.5	5.3	0.44	>100	>227
229	13 14	15 18	11 57	11 ND	>100 >100	> 9 ND
230	1.5	3.8	9.5	1.0	>100	>100
231	0.015	0.15	1.1	0.076	>100	>1 315
232	0,00053 0,00038	0,0096 0,0017	0,0190 0,0041	0,0037 0,0019	5,8 4,5	1 568 2 368

233	1,5 5,4 4,4	13 9,6 11	12 17 15	11 ND 9,7	18 18 22	1,7 ND 2
234	1.5	0.10	0.10	0.95	>100	>105
235	1.6	1.1	0.38	1.2	61	51
236	3.7	8.6	0.12	5.1	>100	>20
237	0.0026	$\leq 0.0001$	0.088	0.0016	18	11,250
238	0.00045	$\leq 0.0001$	0.025	0.0025	59	23,600
239	0.0065	0.00033	0.19	0.0030	20	6667
240	$\leq 0.0001$	$\leq 0.0001$	$\leq 0.0001$	$\leq 0.0001$	2.5	$\geq 25\ 000$



241	0.047	0.17	14	1.4	$\geq 100$	$\geq 74$
242	0.25	0.0010	1.1	0.23	93	404
243	0.0011	0.00050	0.32	0.027	72	2,667
244	1.9	0.019	26	11	$\geq 100$	$\geq 9$
245	$<1E-4$	$<1E-4$	$<1E-4$	$<1E-4$	0.68	$>6\ 800$
246	47	1.4	28	25	$>100$	$>4$
247	0.13	0.00078	0.13	0.10	15	150
249	8.6	0.78	8.4	3.9	$>100$	$>25$

250	0.17	0.16	0.17	0.063	31	492
254	0.17	0.18	0.29	0.098	31	316
256	4.6	5.1	14	5.3	20	4
257	9.7	5	1.6	4.2	>100	>24

5

\*Resistance Factor = Ratio of dCK- on Wild-type CCRF-CEM

ND: Not Determined

**NIH lines:**

**MCF-7: Human Breast Carcinoma**

**H-460: Human Lung Carcinoma**

**SF-268: Human Central Nervous System Tumor**

**CCRF-CEM: T-cell Leukemia**

**Dck-: CCRF-CEM deoxycytidine kinase-deficient**

10

5 Table 2 of IC50 Values ( $\mu\text{M}$ ) for Pro-drugs of BCH-4556

Exposition of 24hr to drug, washed, and incubated for another 48hr  
(total of 72hr assay)

10 IC50  $\mu\text{M}$  (MTT at 72hr) IC50  $\mu\text{M}$  (MMT  
or WST-1 at 72hr)

BCH	H-460 24h	MCF-7 24h	SF-268 24h	CCRF-CEM 24h	CEM/d CK- 24h	Resistance Factor*
Gemcitabine	0,012	0,0060	0,015	ND	>100	ND
	0,017	0,0092	0,064	0,0740	>100	>1 351
	0,086	0,2800	0,180	ND	>100	ND
	0,420	0,2600	0,220	0,0240	6,7	279
	0,046	0,0770	0,056	0,0250	19	760
	0,012	0,1100	0,048	0,0100	49	4 900
	0,086	0,0070	0,270	0,0071	34	4 789
	0,013	0,0150	0,082	0,0067	11	1 642
	0,014	0,0078	0,017	0,0088	56	6 364
	0,012	0,0120	0,840	0,0083	98	11 807
	0,070	0,1200	0,130	0,0051	65	12 745
	0,055	0,0270	0,023	0,0038	>10	>2 631
Average	0,072 $\pm$ 0,1 26	0,078 $\pm$ 0, 107	0,18 $\pm$ 0,25	0,020 $\pm$ 0,023	57 $\pm$ 39	3987 $\pm$ 3871
Cytosine Arabinoside	0,150	0,110	4,1	ND	>100	ND
	0,088	0,058	26	0,0820	>100	>1 220
	0,250	0,510	7,2	ND	>100	ND
	0,780	0,920	73	0,0370	>100	>2 700
	0,130	0,210	39	0,0380	69	1 816
	0,063	0,830	16	0,0130	83	6 385
	0,180	0,054	42	0,0085	15	1 765
	0,081	0,056	15	0,0079	11	1 392
	0,066	0,050	1,9	0,0100	29	2 900
	0,073	0,061	ND	0,0100	69	6 900
	0,350	0,860	7,8	0,0094	91	9 680
	0,095	0,160	5,9	0,0078	>10	>1 282
Average	0,19 $\pm$ 0,22	0,29 $\pm$ 0,3 4	25 $\pm$ 23	0,026 $\pm$ 0,026	68 $\pm$ 36	3135 $\pm$ 2246
BCH-4556	0,35	0,12	16	ND	>100	ND
	0,78	0,63	17	0,44	>100	>227
	3,50	3,20	9,8	ND	>100	ND
	5,10	7,70	45	0,72	>100	>139
	1,70	1,30	15	0,79	>100	>126
	0,51	3,30	32	0,14	>100	>714
	1,30	0,53	28	0,21	>100	>476
	0,76	0,51	19	0,21	10	48
	ND	ND	ND	ND	ND	ND
	0,54	0,72	83	0,14	>100	>714
	2,30	1,60	16	0,16	>100	>625
	0,78	1,50	7,1	0,14	>10	>71
Average	1,6 $\pm$ 1,6	2,0 $\pm$ 2,4	29 $\pm$ 23	0,38 $\pm$ 0,28	>100	349 $\pm$ 283

<b>277</b>	<b>2.0</b>	<b>0.32</b>	<b>7.3</b>	<b>0.48</b>	<b>&gt;100</b>	<b>&gt;208</b>
<b>107</b>	<b>0.27</b>	<b>0.25</b>	<b>3.4</b>	<b>0.024</b>	<b>49</b>	<b>2,042</b>
<b>110</b> (HCl salt: 251)	<b>0,01300</b> <b>0,00049</b> <b>0,00060</b>	<b>0,018</b> <b>0,120</b> <b>0,240</b>	<b>1,10</b> <b>0,14</b> <b>7,50</b>	<b>0,0034</b> <b>0,0025</b> <b>0,0040</b>	<b>1,3</b> <b>7,1</b> <b>9,4</b>	<b>382</b> <b>2 840</b> <b>2 350</b>
<b>172</b>	<b>0,21</b> <b>2,70</b> <b>3,30</b>	<b>0,17</b> <b>1,30</b> <b>0,97</b>	<b>0,76</b> <b>9,70</b> <b>54</b>	<b>0,09</b> <b>0,28</b> <b>0,20</b>	<b>1,3</b> <b>32</b> <b>80</b>	<b>14</b> <b>114</b> <b>400</b>
<b>185</b>	<b>0,86</b> <b>1,70</b> <b>1,80</b>	<b>1,4</b> <b>1,4</b> <b>2,3</b>	<b>4,9</b> <b>5,9</b> <b>17</b>	<b>0,18</b> <b>0,18</b> <b>0,45</b>	<b>12</b> <b>12</b> <b>30</b>	<b>67</b> <b>67</b> <b>67</b>
<b>186</b>	<b>0,0057</b> <b>0,0270</b>	<b>0,047</b> <b>3,4</b>	<b>1,7</b> <b>&gt;10</b>	<b>0,0086</b> <b>0,0790</b>	<b>26</b> <b>14</b>	<b>3 023</b> <b>177</b>
<b>191</b>	<b>≤0,0001</b> <b>0,0078</b> <b>0,0017</b>	<b>≤0,0001</b> <b>0,0041</b> <b>0,0054</b>	<b>0,010</b> <b>&gt;0,1</b> <b>0,065</b>	<b>ND</b> <b>0,0029</b> <b>0,0710</b>	<b>1,1</b> <b>&gt;0,1</b> <b>12</b>	<b>ND</b> <b>&gt;34</b> <b>169</b>
<b>196</b>	<b>0,010</b> <b>0,098</b>	<b>0,0010</b> <b>0,0064</b>	<b>0,045</b> <b>0,650</b>	<b>ND</b> <b>0,010</b>	<b>7,7</b> <b>&gt;1</b>	<b>ND</b> <b>&gt;100</b> <b>43</b>
<b>197</b>	<b>≤0,0001</b> <b>0,0097</b> <b>0,0038</b>	<b>≤0,0001</b> <b>0,00250</b> <b>0,00014</b>	<b>0,01</b> <b>&gt;0,1</b> <b>0,22</b>	<b>ND</b> <b>0,0018</b> <b>0,0530</b>	<b>7,4</b> <b>&gt;0,1</b> <b>&gt;100</b>	<b>ND</b> <b>&gt;56</b> <b>&gt;1 886</b>
<b>198</b> (HCl salt: 261)	<b>≤0,0001</b> <b>0,0062</b> <b>0,0068</b>	<b>0,0001</b> <b>0,0028</b> <b>0,0046</b>	<b>0,0054</b> <b>&gt;0,1</b> <b>0,73</b>	<b>ND</b> <b>0,0083</b> <b>0,1400</b>	<b>10</b> <b>&gt;0,1</b> <b>23</b>	<b>ND</b> <b>&gt;12</b> <b>164</b>

202	≤0,0001 0,021	0,0001 0,0850	0,043 >0,1	ND 0,014	0,05 >0,1	ND >7
203	0,120 0,250 0,050	0,010 0,089 0,120	0,72 >1 7,4	ND 0,010 0,460	1,2 >1 20	ND >100 43
207	0,53 0,65	0,13 0,49	>1 >1	0,074 0,190	>1 >1	>14 >5
208	0,11 0,20	0,031 0,066	0,47 2,20	0,0590 0,0093	25 >1	424 >108
210	0,37 1,70 0,11 0,22	0,130 0,065 0,270 0,110	≥100 >100 51 >100	0,24 0,46 0,13 0,50	51 >100 >100 47	204 >217 >770 94
211 (HCl salt: 248)	0,0053 0,0030 0,0140 ND <1e-6 0,0087	0,00100 0,00015 0,00770 0,00013 <1e-6 0,00130	0,038 0,050 0,034 0,012 0,029 0,034	0,0028000 0,0350000 0,0003300 ND <1e-6 0,0000023	>1 13 >0,1 8,70 1,50 0,44	>357 371 >303 ND >1500000 >191 300
216	0.064	0.0094	0.40	0.34	31	91
217	0.011	0.0039	0.12	0.36	27	75
219	0,014 0,058	0,0037 0,0220	0,18 1,60	0,018 0,010	51 >1	2833 > 100
223	1,70 0,78 4,00	1,7 2,1 1,4	15 47 45	0,12 0,13 0,45	>100 >100 >100	>833 >769 >222

202 203 207 208 210 211 216 217 219 223

226	0,850 0,250 0,065 0,420	0,40 0,26 0,22 0,14	>1 1,8 3,9 17	0,0600 0,0410 0,0011 0,0260	>1 >10 15 35	> 17 >244 13 636 1 346
232	0.0069	0.020	0.16	0.010	2.1	210
237	0,042 5,200 0,170	0,0011 0,0220 0,1700	3,3 1,8 2,7	0,0014 0,0100 0,0040	2,7 22 15	1 928 2 200 3 750
238 (HCl salt: 269)	0,064 0,046 0,017 0,062	0,00460 0,00130 0,00020 0,01000	5,7 1,9 5,6 2,7	0,0170 0,0050 0,0048 0,0014	23 10 5,2 28	1 353 2 000 1 080 20 000
239	0,49 0,20 0,20	0,0021 0,0031 0,6400	9,0 4,9 25	0,0045 0,0022 0,0110	20 28 17	4 444 12 727 1 545
240 (HCl salt: 264)	<1e-6 0,0091 0,0014 0,0069	<1e-6 0,00045 0,00068 0,00190	0,053 0,016 0,031 0,028	<1e-6 0,000011 0,000029 0,000002	1,70 0,11 0,84 1,40	>1 700 000 10 000 28 965 700 000
243 (HCl salt: 260)	0,140 0,038 0,024	0,00640 0,00079 0,12000	14 7,7 68	0,0480 0,0081 0,0400	30 21 51	625 2 593 1 275
245 (HCl salt: 268)	0,00021 0,00290 0,00110	<1E-5 0,00300 0,00013	0,0440 0,0950 0,0047	<1E-5 0,000021 >1E-6	2,2 3,4 6,0	>220 000 161 904 >6E6
247	0,39 0,54 0,46	0,00089 0,30000 0,01600	6,1 >10 14	0,024 0,140 0,170	61 49 61	2 542 350 359
257	89 42	36 21	>100 >100	4,1 5,4	>100 >100	>24 >19

262	0.90	16	>100	0.88	>100	>114
263	66 >100	73 12	>100 >100	19 14	>100 >100	>5 >7
265	>100	77	>100	30	>100	>3
266	0,00690 0,00053	0,0120 0,0013	1,00 0,42	0,00190 0,00067	21 26	11 050 37 143
267	93	34	>10	2.9	>10	>3

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10 The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

15 From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention and, without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

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